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Prenatal Evaluation of Growth by Ultrasound

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Abnormal growth patterns are associated with an increased risk of perinatal morbidity and mortality. Therefore, the ability to evaluate growth at a very early age in utero utilizing ultrasound techniques is a major advancement in minimizing fetal and perinatal morbidity and mortality.¹ Understanding the applications of ultrasound to evaluate normal and abnormal intrauterine growth enhances significantly the understanding of postnatal growth. Unfortunately, pediatricians have had limited opportunity to become acquainted with these applications. The purpose of this article is to help fill that void.

Prenatal growth is divided into 2 periods: embryonic and fetal. Prenatal ultrasound allows specific measures of both embryonic and fetal structures, and comparison to normal values permits evaluation of

In This Issue

Letter From the Editor pg 4

The Use of Fluorescence In Situ Hybridization to Identify Human Chromosomal Abnormalities pg 6

Genetics Glossary Insert

Abstracts pg 12

growth patterns. Prenatal evaluation is usually possible 3 weeks postconception.

EMBRYONIC DEVELOPMENT AND GROWTH

Embryonic development and growth starts with fertilization and progresses through blastogenesis (postconception days 0 through 21) and organogenesis (days 21 through 60). In humans, fusion of the eyelids (days 56 through 60) is regarded as an arbitrary end of the embryonic period (Table 1).²

Table 1: Human Embryonic Development and Growth

PERIOD	CONCEPTION [†] (d)	LMP [‡] (d)	CROWN-RUMP LENGTH (mm)	EXTERNAL CHARACTERIZATIONS	Staging [§]	
					Jirasek	Streeter [*] Carnegie ^{**}
Blastogenesis	0-14	0-28	0-0.4	Unicellular to bilaminar plate	1-4.3	1-6b [*]
	15-21	29-35	0-4.2	Trilaminar embryo to open neural groove	5.1-6.1	7-8 [*] ix-x ^{**}
Organogenesis	22-35	36-49	2-8	Neural tube closure to limb buds	6.2-7.2	xi-xiii ^{**}
	36-60	50-74	8-35	Limb growth to fused eyelids	7.3-8.2	xiv-xxii ^{**}
Fetal	61-266	75-280	35-350	Fetal maturation	9-10	-

[†]Embryonic development is dated from conception.

[‡]Prenatal growth evaluation by ultrasound is dated from day of last LMP (last menstrual period). This is termed "gestational age."

[§]Adapted from Jirasek JE. In: Sciarra JJ, ed. *Gynecology and Obstetrics*. Philadelphia, Pa: Lippincott Co; 1991:2:1.

While embryonic development is dated from conception, prenatal growth evaluation by ultrasound is dated from the first day of the last menstrual period, which is termed "gestational age," and this is the term and time relationship used subsequently in this article.

Normal embryonic and early fetal growth occurs spontaneously and is not affected by secondary factors such as uterine size and placental function, as is growth in the second and third trimesters. Normal growth³ in the last 2 trimesters is roughly linear, with some slowing from approximately 38 weeks gestation until delivery. However, this slowing may start earlier in twin gestations and in different ethnic populations. Following this normal deceleration in late pregnancy, there is an acceleration after birth and growth similar to in utero growth during the second and third trimesters. Normal embryonic and fetal growth allows an embryo weighing approximately 2 g at 7 to 10 weeks to grow to a fetal mean weight of 3240 g at 42 weeks.

PRENATAL EVALUATION OF GESTATIONAL AGE

In order to evaluate prenatal growth, it is necessary to accurately estimate the gestational age of the embryo and fetus. Ultrasound measurements used for this estimation are: the mean gestational sac diameter; the crown-rump length (CRL); the biparietal diameter (BPD); and femur length (Table 2).

A gestational sac⁴ can usually be identified at 5 weeks and is an early indication of an intrauterine pregnancy. A gestational sac does not confirm a viable pregnancy. Ultrasound evaluation of the embryo can be summarized as follows⁵:

1. At 6 weeks gestational age, embryonic structures and heart activity are almost always visible.
2. At 7 weeks, the embryo is 10 mm at a minimum and fetal heart activity should be visible in 100% of viable pregnancies.

3. At 8 weeks, fetal structures are visible and the yolk sac is identified as a circular structure measuring 5 mm in diameter. The detection of a yolk sac excludes the diagnosis of a blighted ovum since a viable embryo is necessary for yolk sac development.
4. An empty gestational sac with a mean diameter greater than 30 mm with no visible embryonic structures means a nonviable pregnancy (blighted ovum) exists.
5. At 9 to 11 weeks, progressive ossification occurs with major centers in the calvaria and ilium.

The CRL is measured from the outer edge of the cephalic pole to the outer edge of the fetal rump. This measurement predicts the gestational age with an error of ± 3 days (90% confidence limits) after 7 to 10 weeks. The error increases to ± 5 days between 10 and 14 weeks gestation. Fetal flexion may decrease maximal CRL length by 5%.

The BPD is maximally accurate for estimation of gestational age between 12 and 20 weeks. It is measured at the level of the thalamus from the outer table of the proximal skull to the inner table of the distal skull. Changes in skull shape, ie, flattening or rounding, can be identified by the cephalic index (CI).⁶ This is the ratio of the BPD divided by the occipital frontal diameter (OFD). A normal ratio is 0.75 to 0.85. After 20 weeks gestation, the BPD is less reliable for gestational dating due to changes in shape, growth disturbances, and individual variation.

Femur length is an excellent parameter to determine fetal age. The femur can be measured as early as 10 weeks gestational age.⁷ Normal percentile charts are available for the femur and for other long bones, including the humerus, ulna, radius, tibia, and fibula.⁸

Fetal BPD and femur length for gestational age dating have a confidence interval of ± 1 week from 12 to 22 weeks, ± 2 weeks from 22 to 32 weeks, and ± 3 weeks from 32 to 41 weeks.

Table 2: Measurements of Gestational Age by Various Parameters

MEAN GESTATIONAL AGE (wk)*	MEAN GESTATIONAL SAC DIAMETER (mm)**	EMBRYO CROWN-RUMP LENGTH (mm)	FETAL BIOMETRY BIPARIETAL DIAMETER (mm)	FETAL BIOMETRY FEMUR (mm)†
5+0	2	-	-	-
6+0	10	6	-	-
7+0	18	10	-	-
8+0	26	17	-	-
9+0	6	25	-	-
10+0	-	33	-	-
11+0	-	43	-	6
12+0	-	55	17	9
13+0	-	68	20	12
14+0	-	85	25	15

*From 1st day of last menstrual period

**Daya et al, 1991

† Jeanty, 1983

OTHER FACTORS TO BE CONSIDERED IN THE PRENATAL EVALUATION OF FETAL GROWTH

The accuracy of gestational age estimation using ultrasound needs to be considered. In determining this, one must understand that all parameters used to measure fetal growth have ranges of normal values, which vary with gestational age. It also is important to have an appropriate set of normal data for the population under study. Establishment of one's own normal data base for comparison with published standard curves is highly desirable. At a minimum, each reproductive center must have collected enough data known to be accurate to ascertain that the published data apply to the use of their equipment, technology, and experience.

Birth weight distribution is non-Gaussian as the birth weight of a significant portion of newborns falls outside the expected distribution. Small for gestational age (SGA) is defined as a birth weight less than the 10th percentile. Therefore, the SGA group includes a majority of normal but small infants. Accelerated catch-up growth in the first 3 months of life usually occurs if intrauterine growth problems are independent of fetal genetic factors. Unfortunately, this postnatal observation is not available during prenatal assessment when a fetus is SGA. Large for gestational age (LGA) is defined as a birth weight greater than the 90th percentile. This LGA group also will include normal but large infants.

Fetal weights can be estimated by using established charts comparing the BPD and the abdominal circumference (AC).^{9,10} Alternatively, the head circumference (HC) can be compared with the AC. The HC exceeds the AC until 38 weeks gestation, when they become equal. The AC subsequently exceeds the HC. Estimations of fetal weight also can be obtained by using average fetal size percentiles and comparing these measurements to a birth weight-gestational age table. These birth weight-gestational age tables will vary for different populations. The estimated fetal weight using BPD and AC has an error range of approximately $\pm 15\%$.⁹ There is a tendency to overestimate fetal weights, and the estimate is less accurate when fetal weights are more than 4000 g. The accuracy of the estimated fetal weight is improved in the SGA fetus below the 5th percentile. The calculated weight estimation has decreased sensitivity but fewer false-positive results. Differences in fetal sex also will influence fetal weight. Females have a 3% to 8% lower weight at the same gestational age. Race and parity also may affect normal fetal growth.

The use of BPD and AC gives only minimally greater accuracy than using AC alone, but there is a much higher predictive value in a growth-retarded population.¹¹

AC measurement correlates most strongly with overall size of the fetus.¹² The AC is a cross-sectional measurement of the upper fetal abdomen at the level of the liver (fetal portal venous system) and stomach.

The fetal liver is relatively large and significantly affected by growth retardation. However, the AC estimates only whether the fetus is large or small. The sensitivity and predictive value of a positive test increases with gestational age. Optimal screening is at gestational age 34 ± 1 week.

Age-independent ratios also have been considered. Femur length divided by AC after 24 weeks remains at 0.22 ± 0.02 .^{13,14} This is more useful when accurate gestational age is unknown. The ratio for growth retardation is greater than .235; for macrosomia the value is less than .205.

Another age-dependent ratio is HC:AC, which may allow classification into symmetric and asymmetric growth patterns, if HC or AC is outside the normal range.

Recommendations^{15,16} for ultrasound measurements of fetuses at risk for growth abnormalities usually include dating the pregnancy at 10 to 12 weeks. This is followed with serial growth assessment by ultrasound every 6 weeks between 18 and 30 weeks, and every 3 weeks from 30 weeks to delivery. Longitudinal studies in fetuses at risk for growth retardation indicate that the growth pattern in affected fetuses is more erratic than that seen in normal pregnancies, and may show periods of normal and abnormal growth rates. As the growth problem becomes more serious, the abnormal growth rate persists.

In general, multiple abnormal parameters indicate a more serious problem than a single abnormal parameter, and the risk for a bad outcome significantly increases when the weight estimate and/or HC become abnormal.

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Letter From the Editor

Dear Colleagues:

In 8 years of publication of *GROWTH, Genetics, & Hormones*, the Editorial Board has been instrumental to the successes of the publication. Editorial Board Members have been picked because of their capabilities as teachers, communicators, authors, and investigators. In addition to those who currently serve, Dr. William Clarke, Dr. Judith Hall, Dr. William Horton, Dr. Jean-Claude Job, and Dr. Fima Lifshitz, whose capabilities and participation I admire and appreciate, there are others who have rotated off the Board and deserve equal recognition. These are Dr. Jürgen R. Bierich, Dr. David L. Rimoin, Dr. Alan D. Rogol, and Dr. James M. Tanner.



Dr. Allen W. Root, Professor of Pediatrics and Molecular Biology, All Children's Hospital, University of Florida, joined the Board on January 1, 1993. He is eminently qualified as he has broad academic and practical experience in pediatric endocrinology and has written extensively in leading journals and textbooks for almost 30

years. Dr. Root's acceptance of an appointment to the Editorial Board will unequivocally enhance the quality of *GROWTH, Genetics, & Hormones*.

Sincerely,
Robert M. Blizzard, MD

Letter To the Editor

This letter is written in follow-up to the abstract and editorial comments concerning our paper entitled "Psychosocial Growth Failure: A Positive Response to Growth Hormone and Placebo" published in *GROWTH, Genetics, & Hormones*, Vol. 8, No. 4.

I welcome the opportunity to elaborate on the characteristics of the children who were included in our study and who had psychosocial growth failure (PSGF) or psychosocial short stature (PSS). I will comment regarding the differences between these patients and those you have described with PSS.

We have a steady referral of classic cases of emotionally damaged children (often with physical abuse) who have all the textbook features of PSGF with impaired growth hormone (GH) secretion, who bounce back to normal once they are placed in foster care. This type of case was excluded from those presented in our article which Dr. Clarke abstracted for *GGH*.

The children in this series were typical short, slow-growing children who had normal GH testing during both sleep and pharmacologic stimulation.

The psychologic assessment revealed anxious attachment. Their depressive symptoms were typical: acting out and/or withdrawal, sad affect, and oversensitivity to minor crises.

They *did not show* lack of discrimination in relationships, nor did they display the self-destructive behavior, pain agnosia, or bizarre eating and sleeping disorders seen in classic PSS. In addition, the parents were not indifferent and rejecting, as are those of patients with typical PSS. The parents had insight into their problem, and several felt guilty and/or had depression. In the classic PSS situation, as you and your colleagues have described it, the parents are

usually considered untreatable because of their own personality damage and entrenched rejection of the child.

The children underwent a battery of psychologic instruments. These included ratings for depression, from which the results were added to those of the diagnostic interview. The assessment process was intense, focusing in large part on relationships with the family. The individual assessments allowed many of the children to discuss concerns that had not previously surfaced. These factors, together with the relative emotional health of the parents in our group, appeared to generate a therapeutic effect from the assessment process itself. This was not predicted when the study initially began. Anecdotally, the parents reacted much more favorably to their children; they all reported greater appetite, even though objective measurements discounted that! This positive outcome remained.

This level of attachment disorder is probably common, underrecognized, and well-camouflaged in middle-class families. Its identification needs an independent psychiatric evaluation, separate from the child's own specialist. I recognize that many people will remain skeptical about the diagnosis of depression in short children. However, the placebo effect was a real surprise. I had not wanted to include it. The Ethics Committee recommended it, and the results are hard to explain, apart from a major change in family function.

I certainly do not recommend GH (or placebo injections!) be used as a cure-all. In fact, our rate of new cases undergoing GH therapy has leveled off in the past year or 2. We have 65 children on GH, for a referral population of 110,000 children aged 0 to 16 years. However, I do think that these observations are a salutary reminder that even if we often deal with

rare and complex diseases with expensive treatment, family issues are the bread and butter of pediatrics, and close collaboration with our liaison child-psychiatrist colleagues can help identify those parents and children who need more expert help.

Yours sincerely,

T.J.C. Boulton, MD
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Editor's comment: Dr. Boulton was very kind to write and clarify the characteristics of the patients reported in the paper written by him and his colleagues (abstracted in GGH 1992; 8[4]:13). The observation that children with nonclassic PSGF may respond to GH, and to some extent placebo injections, is most interesting and important.

The description of the patients and their parents reported by Boulton et al requires emphasis that there are several different types of PSS. I suggest that we now use 3 different groupings for PSS, specifically:

Type 1 - Children under 2 years of age whose mothers are overburdened with responsibilities, often neglectful, and who fail to provide adequate calories and stimulation. These patients secrete GH and

respond to increased caloric intake with increased growth. Nutritional failure is the primary etiology of growth failure in this group (Krieger I. Clinical Pediatrics 1974;13:127-133).

Type 2 - Children 2 years of age or older whose parents psychologically abuse and reject them. The majority of these children have diminished GH secretion and some or all of the classic characteristics—polyphagia, polydipsia, encopresis, severe shyness or aggressiveness, pain agnosia—and may or may not be underweight for height (Powell GF et al. N Engl J Med 1967;276:1271-1283. Blizzard RM, Bulatovic A. Psychosocial short stature: a syndrome with many variables. In: Bierich J, ed. Baillieres' Clinical Endocrinology and Metabolism. Volume 6, Number 3. Philadelphia, Pa: WB Saunders Co [Bailliere Tindall Ltd]; 1992.) These children have many physical characteristics in common with GH-deficient patients.

Type 3 - Children over 2 years of age who are depressed, whose parents are not rejecting but who may have emotional problems themselves. The children do secrete GH in normal amounts, but otherwise have many characteristics in common with GH-deficient patients (Boulton TJC et al. Acta Paediatr Scand 1992;81:322).

May I also suggest that we will broaden the spectrum of PSS as our knowledge increases.

Robert M. Blizzard, MD

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by Kenneth L. Jones, MD

Adrenarche and Its Variants

by Songya Pang, MD

The Importance and Methods of Using Animal Models to Study Human Disease

by Robin Winter, MD

Contiguous Gene-Deletion Syndromes

by Frank Greenberg, MD

Clinical Significance of Urinary GH Measurements

by Margaret MacGillivray, MD

The Use of Fluorescence In Situ Hybridization to Identify Human Chromosomal Anomalies

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During the 20 years since the initial discovery of chromosome banding,* the clinical relevance of chromosomal analysis has become firmly established for genetic diagnosis and evaluation of tumor-specific chromosomal alterations. Global surveillance of the entire cellular genome* by routine and high-resolution cytogenetic studies permits detection of numerical and structural chromosomal abnormalities, allowing the visual diagnosis of alterations of single chromosomal bands on the order of approximately 5 to 10 million base pairs.* Smaller changes, those involving less than 1 million and up to several million base pairs of DNA, are difficult or impossible to detect using standard cytogenetic methods. Chromosomal alterations with indistinct banding patterns, such as marker chromosomes,* de novo unbalanced translocations,* and abnormally banded regions in somatic* or tumor cells, also are a problem to identify. Other drawbacks to standard cytogenetic techniques arise from the fact that to perform chromosome analysis the cells must be dividing and the chromosomes must be arrested in metaphase. This means that the process requires a significant investment of time and labor to generate enough dividing cells to perform the study. Finally, cell selection may occasionally cause the results of such studies to be misleading because cells that proliferate in vitro may not be representative of the original population. This is particularly a problem when dealing with tumor specimens.

Many of the aforementioned difficulties have been circumvented with the introduction of fluorescence in situ hybridization* (FISH) technology into clinical diagnostic laboratories. This relatively new technology provides an important adjunct to classic cytogenetics because of its unique ability to simultaneously assess molecular and cytologic information. This has led directly to numerous clinical applications, such that FISH methods have been developing at a rapid pace for the purposes of high-resolution gene* dosage analysis and chromosomal abnormality detection.

The ability to detect and characterize chromosomal abnormalities using FISH has been greatly enhanced by the rapidly increasing availability of numerous

chromosome-specific probes.* In addition, it is possible to accomplish a diagnostic assay within 24 to 48 hours, in contrast to the greater amount of time required for some cytogenetic analyses or for performing standard Southern blotting* to assess DNA sequence copy number. Several cytogenetically-based disorders are more easily assessed by FISH than by other routine cytogenetic studies. The recent studies by Kuwano et al, Altherr et al, Goodship et al, and others suggest that submicroscopic or cryptic translocations, which occur in association with the Miller-Dieker and Wolf-Hirschhorn syndromes, for example, are better assessed with FISH because there is more complete diagnostic capability than with other techniques. This allows more accurate determination of risks of recurrence of a defect.

FLUORESCENCE IN SITU HYBRIDIZATION

FISH permits determination of the number and location of specific DNA sequences* in human cells (Table 1A), both in interphase nuclei and directly on metaphase chromosomes. The FISH procedure relies on the complementarity between the 2 strands of the DNA double helix. Probe DNA molecules are nonisotopically labeled by incorporation of a chemically modified nucleotide that is subsequently detected with a fluorescently

Table 1
FISH Assays

A) Permit

- Copy number of specific chromosomes (aneuploidy detection)
- Copy number of specific chromosomal regions (duplication or deletion detection)
- Identification of unknown or derivative chromosomes
- Analysis and diagnosis of nonrandom chromosomal translocations

B) Procedure Steps

- Label the probe DNA
- Prepare and denature the sample or target DNA (metaphase chromosomes or interphase nuclei)
- Hybridize the denatured, single-stranded probe to denatured target DNA
- Wash away unbound or weakly homologous labeled probe DNA
- Detect the resulting probe DNA:target DNA hybrid molecules

*Please reference *Genetics Glossary* insert.

tagged reporter molecule (Table 1B). Most often, nucleotides* substituted with biotin-dUTP or digoxigenin-dUTP are enzymatically incorporated by nick-translation* into probe DNA in place of thymine. These are usually employed because of the high sensitivity of DNA-DNA hybrid detection and the commercial availability of the appropriate labeling and detection reagents. Taking advantage of the biotin-avidin affinity reaction, biotin can be detected with fluorescently tagged avidin, whereas digoxigenin is detected with fluorescently tagged antidigoxigenin antibodies.

The target cells or metaphase chromosomes to be tested are prepared on glass microscope slides. The cells are usually derived from specimens similar to those that would normally be employed for standard cytogenetic studies. Metaphase spreads from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes or from dividing cells obtained from bone marrow aspirates or from cultured cells recovered from skin or tumor biopsy specimens are usually used. Specimens appropriate for prenatal diagnosis can be retrieved from amniotic fluid, chorionic villi, or fetal blood obtained by fetoscopy or percutaneous umbilical blood sampling (PUBS). However, and most importantly, FISH does not require preparation of metaphase chromosomes to be successful, in contrast to more standard cytogenetic approaches. Hence, for rapid diagnosis, as would be preferred for prenatal and tumor specimens, one has the option of directly fixing and immobilizing interphase nuclei of nondividing cells retrieved directly from the tissue source or even tissue sections. Since each individual chromosome occupies a moderately discrete territorial domain within the interphase nucleus,* it is possible to determine chromosomal or regional copy number by counting the number of signals present. Thus, using the immobilized interphase nuclei as the hybridization* target, one is able to diagnose chromosomal abnormalities. This approach, termed interphase cytogenetics, speeds diagnosis and avoids the problems encountered with cell selection upon extended cell growth.

The probe and target DNAs are denatured, and the modified probe DNA is hybridized to the target at a temperature below the melting point of the DNA duplex. This allows the modified probe to bind to its complementary sequences in the target. Many DNA probes contain repetitive DNA sequences, of which there are numerous copies throughout the genome. Thus, to suppress nonspecific hybridization or binding to the repetitive sequences in the target DNA, the repeat sequences in the probe DNA are blocked by addition of competitor DNA to the hybridization mixture. This is accomplished by the addition of either total genomic* DNA or Cot-1 DNA, which is a DNA fraction selectively enriched in highly repetitive sequences. Probes are

hybridized for 3 to 18 hours, and then unbound probe is washed off the target, and fluorescent detector molecules are added to and combine with the modified probe hybridized to the specimen. Fluorescent detection is most often accomplished with conventional epifluorescence microscopy; if, however, the fluorescent signal is weak, digital imaging techniques can be used.

PROBES

Several different types of probes are available for use in the detection of chromosomal abnormalities. The choice of probe will vary with the particular application in question. In general, probes fall into 3 classes: (1) locus*-specific probes; (2) alphoid or centromeric repeat probes; and (3) whole chromosome or chromosomal region "painting" probes. In addition, total genomic DNA can be used as a probe to determine the human component in human/rodent somatic cell hybrids* (Figure 1A, page 8).

Locus-Specific Probes: Applications directed at aneuploidy* detection, which is detection of duplication or deletion of specific chromosomal regions, and detection of tumor-associated translocation breakpoint detection rely on the availability of probes that are specific for and that reliably generate a bright fluorescent signal at a unique chromosomal locus in metaphase or in an interphase nuclei. These DNA probes range in size from 15 to 500 kilobases* and are used to diagnose a specific cytogenetic abnormality. They are generated by identification and propagation of a locus-specific DNA segment cloned into a large insert phage,* cosmid,* or yeast artificial chromosome (YAC)* vector. For example, hybridization signals seen on the metaphase chromosomes from a normal individual after hybridization with 2 unique cosmids that map to chromosome 22, 1 to the proximal long arm and 1 to the distal long arm, are demonstrated in Figure 1B (page 8). Figure 2 (page 9) also is a diagrammatic representation of a single-locus probe hybridization for aneuploidy detection. Numerous locus-specific markers are becoming readily available for significant chromosomal loci as a result of the intensive mapping efforts under way in association with the Human Genome Project.*

Alphoid or Centromeric Repeat Probes: Satellite or repetitive DNAs constitute approximately 10% to 20% of all human DNA. In particular, chromosomes carry from 10^5 to more than 10^6 base pairs of centromeric and pericentromeric short, tandemly repeated DNA sequences. The monomeric units that form these alpha satellite, beta satellite, or other satellite DNA sequences vary, such that many are chromosome-specific. Hence, chromosome-specific, repeat sequence probes have been isolated and cloned for the majority of human chromosomes, and many of these

probes are commercially available. These repeat probes produce signals that are very intense in interphase nuclei and on metaphase chromosomes (Figures 1C, 1D, and 2).

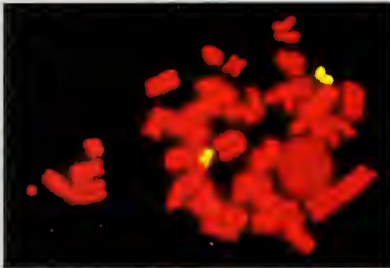
Whole Chromosome (Painting) Probes: FISH using a probe mixture composed of numerous different DNA sequences with homology to many sites along a single chromosome permits the entire chromosome to be "painted" or "decorated" both in metaphase or in the interphase nucleus. Painting probes have been produced from chromosome-specific, flow-sorted libraries*

and by polymerase chain reaction (PCR)* amplification* of DNA derived from either monochromosomal somatic cell hybrids or flow-sorted chromosomes. Another approach to preparation of chromosome- or region-specific probes has been microdissection of chromosomes or chromosomal regions from metaphase spreads followed by PCR amplification of the microdissected material (Meltzer et al, 1992). This PCR-amplified material can then be painted back to normal metaphase chromosomes to determine the origin of the microdissected material.

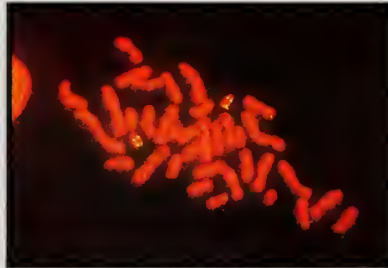
Figure 1

Fluorescence In Situ Hybridization

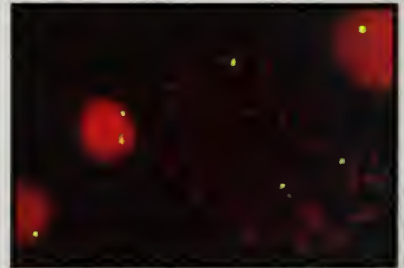
Shown below are the results of FISH using probes of different degrees of complexity. In all cases, chromatin is counterstained with propidium iodide (red fluorescence). The target regions, hybridized with biotinylated probe DNA, are detected with fluorescein isothiocyanate (FITC)-avidin, which appears with yellow fluorescence in these photographs.



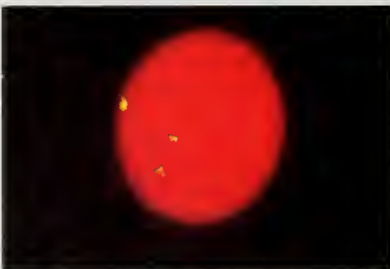
(A) A metaphase spread from a human X hamster somatic cell hybrid (EYEF3A6) with chromosome 22 as its major human component. The metaphase is hybridized with total human genomic DNA, which identifies the human material in the metaphase spread. Chromosome 22 is seen as an intact brightly fluorescent chromosome and there is a fragment of another human chromosome translocated to a hamster chromosome.



(B) Hybridization of metaphase chromosomes from a normal male with 2 labeled cosmid probes (~40 kb/probe). The cosmids recognize the DiGeorge critical region (DGCR) near the centromere and a locus on the distal long arm of the chromosome.



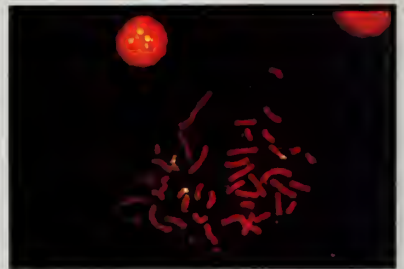
(C) Metaphase spread and interphase nuclei from a patient with karyotype of 46,XX/47,XX,+r. Phenotypic features were consistent with mosaic trisomy 8. Hybridization with the chromosome 8 centromere-specific probe (ONCOR; Gaithersburg, Md) demonstrates the presence of 3 discrete signals in metaphase spreads containing the ring chromosome and mosaicism for the ring in interphase nuclei.



(D) Interphase nucleus from a pediatric glioma hybridized with a chromosome 7 centromere-specific probe (ONCOR; Gaithersburg, Md), demonstrating the presence of trisomy for chromosome 7. The aneuploidy was not detected in metaphase chromosome spreads.



(E) Microdeletion detection in a patient with velocardiofacial syndrome using the cosmids described in Panel B (above center). One of the chromosome 22 homologues is positive with both probes, whereas the deleted chromosome is labeled only for the probe at the telomere.



(F) Metaphase spread from a patient with karyotype of 46,XX,15p+. Parental chromosomes were normal. The 15p+ chromosome was distamycin-DAPI-negative and centromere 15 probe-negative, suggesting the centromere was not from chromosome 15. The G-band pattern resembled 17p, and the Miller-Dieker syndrome probe cocktail, which labels the centromere of chromosome 17 and 17p13 (ONCOR; Gaithersburg, Md), was hybridized to metaphase spreads. Fluorescent signal is detected on 3 chromosomes, the 2 normal 17s and the 15p+.

GROWTH

Genetics & Hormones

Dear Colleagues:

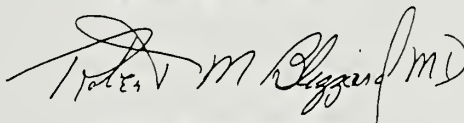
The field of genetics is where the action is today. The members of the Editorial Board have been aware for some time that the fields of genetics, pediatric endocrinology, nutrition, and growth are intimately intertwined. This knowledge prompted establishment of *GROWTH, Genetics, & Hormones* to stimulate and facilitate intellectual exchange of important knowledge among these disciplines. Drs. William Horton and Judith Hall have been key in representing members of the genetic subspecialty on our Editorial Board. The glossary that you are holding in your hands results from their efforts to simplify and interpret terms that recently have appeared in the vocabulary of geneticists. This they have done to permit us to more readily understand that which we read. We thank them for their effort and contribution.

We also thank Genentech, Inc. for the additional funds placed in our educational grant so this glossary can be brought to you.

Please note that this glossary is physically separate from the remainder of the publication. This is by design to permit you to readily access the information in the glossary when you need it in interpreting the articles that you will read in the future, both in *GROWTH, Genetics, & Hormones* and elsewhere.

We hope this endeavor constructively assists you in quickly understanding more fully the important and pertinent articles that will be appearing in future issues of *GROWTH, Genetics, & Hormones*.

Respectfully,
For the Editorial Board

A handwritten signature in black ink, appearing to read 'Robert M. Blizzard MD', with a stylized flourish at the end.

Robert M. Blizzard, MD
Editor

GROWTH

Genetics & Hormones

GENETICS GLOSSARY

allele An alternative form of a gene at a given locus. Being diploid organisms, humans may have 2 alleles at a given locus, ie, a normal and a mutant allele.

allelic disorders Disorders, which may be phenotypically different, that are due to mutations in the same gene.

Alu repetitive sequence Repetitive sequence found about 500,000 times in human genome. The sequence contains a recognition site for the restriction enzyme *Alu* I and is around 300 base pairs in length.

amplification An increase in the number of copies of a particular DNA fragment. Can occur under natural circumstances, eg, amplification of a repeat sequence as in fragile X syndrome, or during laboratory procedures such as cloning or polymerase chain reaction.

annealing See hybridization.

aneuploid A chromosome number that is not an exact multiple of the haploid number. Usually refers to an absence (monosomy) or an extra copy (trisomy) of a single chromosome.

anticipation Phenomenon in which the severity of a genetic condition appears to become more severe and/or arise at an earlier age with subsequent generations.

antisense strand (of DNA) The noncoding strand of the DNA double helix that serves as the template for mRNA synthesis.

autosome Any chromosome other than the X or Y. Humans have 22 pairs of autosomal chromosomes.

bacteriophage (phage) Bacterial virus used as a vector for cloning segments of DNA.

band (chromosomal) A chromosomal segment defined by staining characteristics. Both lighter and darker segments are called bands, and are numbered from the centromere outwards, with smaller bands classified by a second number. Example - (p=short arm; q=long arm; ie, 3p25=short arm of chromosome 3, second band out from the centromere, fifth band outward within the second large band.)

base pair (bp) In the DNA double helix, a purine and pyrimidine base on each strand that interact with each other through hydrogen bonding. The number of base pairs is often used as a measure of length of a DNA segment, eg, 500 bp.

base sequence The order of nucleotide bases in a DNA molecule. Length is usually defined as the number of base pairs.

breakpoint Refers to sites of breakage when chromosomes break (and recombine).

CCAAT box Sequence that occurs 70 to 90 base pairs upstream from the initiation start site of a gene. The sequence is thought to be involved in regulation of transcription.

***Caenorhabditis elegans* (*C. elegans*)** Round worm used as an experimental model, especially in developmental biology.

carrier A clinically unaffected individual who may have clinically affected offspring. The term traditionally refers to an individual who is heterozygous at a given autosomal locus for a normal and a mutant gene (which causes disease only in the homozygous state) or a female who is heterozygous at an X-linked locus for a normal and a mutant gene (which causes disease in the hemizygous state in males). More recently used to describe unaffected individuals who carry unstable or dynamic mutations that can expand and cause a genetic condition in offspring.

cDNA Most often implies (complementary) DNA synthesized from RNA that corresponds to expressed sequences of genomic DNA. The term complementary DNA also may refer to DNA that is complementary to a particular DNA sequence.

cDNA library A collection of clones containing inserts of cDNA fragments representing expressed sequences (mRNA). cDNA libraries differ from 1 tissue or cell type to another.

centimorgan (cM) Measure of genetic distance defined in terms of recombination frequency. Two genetic loci are 1 cM apart if there is a 1% chance of recombination due to crossing over in a single generation. In humans, 1 cM corresponds to approximately 1 million base pairs.

centromere A specialized chromosome region to which spindle fibers attach during cell division. Appears as a distinct "waist" by microscopy.

chimera An organism comprised of cells from 2 or more zygotes.

chorionic villus sampling (CVS) Procedure used to obtain fetal cells for prenatal diagnosis; involves biopsy of the placental membranes.

chromatid Chromosomal strands produced during meiosis when a chromosome divides.

chromatin The composite of DNA and proteins that comprise chromosomes.

chromosome A highly ordered structure composed mainly of chromatin that resides in the nucleus of eukaryotic cells.

cis (1) Historically implies on the same chromosome. (2) In molecular biology refers to an effect on a gene directed by the sequence of that gene in contrast to trans effects, which are produced by other factors, such as transcription factors encoded by other genes. The terms are commonly used to describe factors that influence gene expression.

cloning (1) Production of genetically identical cells (clones) from a single ancestral cell. (2) Cloning is utilized in molecular biology to propagate single or discrete DNA fragments of interest.

cloning vector A DNA segment capable of autonomous replication, ie, a plasmid or phage, that is used to carry the desired DNA segment for replication.

codon A triplet of bases in DNA or RNA that specifies a single amino acid.

codon usage Given the degeneracy of the genetic code, refers to the preference of codons used to specify particular amino acids. Often differs among species and among different genes and proteins.

compound heterozygote An individual who has 2 different mutant alleles at a given locus.

consanguinity Relationship of 2 individuals by descent from a common ancestor. A consanguineous mating is one in which the mates are related, ie, first cousins.

consensus sequence A minimum nucleotide sequence found to be common (although not necessarily identical) in different genes and in genes from different organisms that is associated with a specific function. Examples include binding sites for transcription factors and splicing machinery.

conserved sequence Base sequence in a DNA molecule (or an amino acid sequence in a protein) that has remained essentially unchanged throughout evolution.

contig map Genetic map showing the order of (contiguous) DNA fragments in the genome.

contiguous gene syndrome Syndrome due to abnormalities of 2 or more genes that map next to each other on a chromosome; most often caused by a deletion that involves several contiguous genes.

cosmid Vector used to clone moderate-sized fragments of DNA (up to 45 kilobases). See plasmid.

CpG island Short DNA sequence having a high content of cytosine and guanine (CG) dinucleotides. CpG islands are often found near the transcription start sites of genes.

CVS See chorionic villus sampling.

degeneracy (of the genetic code) Different codons code for the same amino acid.

DNA (deoxyribonucleic acid) The polymeric, double-stranded molecule that encodes genetic information. The strands are held together by hydrogen bonds between nitrogenous bases that constitute the code: adenine (A) and thymine (T), which pair with each other, and guanine (G) and cytosine (C), which pair with each other.

DNA marker A DNA sequence variation that is easily detectable; examples include restriction fragment length polymorphisms, dinucleotide and trinucleotide repeat polymorphisms.

DNA methylation Attachment of methyl groups to DNA, most commonly at cytosine residues. May be involved in regulation of gene expression. Also may prevent some restriction endonucleases from cutting DNA at their recognition sites.

DNA polymerase Enzyme responsible for replication of DNA.

DNA sequence The relative order of base pairs.

domain A discrete portion of a protein (and corresponding segment of gene) with its own function. A protein may have several different domains and the same domain may be found in different proteins.

dominant mutations Mutations that produce an abnormal clinical phenotype (disorder or trait) when present in the heterozygous state.

dominant negative mutations Heterozygous mutations in which the product of the mutant allele interferes with the function of the product of the normal allele.

downstream A DNA sequence is written from the left, or 5', direction or to the right, or 3', direction. Downstream refers to the 3' direction, ie, the stop codon for a gene is downstream (3') of the coding sequences of that gene.

***Drosophila melanogaster* (*drosophila*)** Fruit fly used for classic genetics studies and utilized as an experimental model by developmental biologists.

dysmorphology Study of abnormalities of morphologic development.

electrophoresis An analytical method used to separate nucleic acid, peptide, or protein fragments based on size and charge of the molecule; typically smaller fragments travel farther through the media (gel) in which separation is carried out.

enhancers DNA sequences that increase transcription of a nearby gene; they can act in either orientation, may be either upstream (5') or downstream (3') to the gene or within an intron.

***Escherichia coli* (*E. coli*)** Common bacterium extensively used in cloning.

euchromatin The chromatin that is thought to contain active or potentially active genes. Light (vs dark) bands on G-banding.

exons The sequences within a eukaryotic gene that code for protein, in contrast to introns, which do not.

F1, F2, etc The first (F1), second (F2), etc, generations of progeny of a mating.

FISH See fluorescence in situ hybridization.

fluorescence in situ hybridization (FISH) A physical mapping technique in which fluorescein-tagged DNA probes are hybridized to chromosomes.

fragile site Gap or defect noted in the continuity of a chromosome when stained, eg, fragile X site. Many are apparent only when cells are cultured under special conditions.

frameshift mutation A mutation that alters the normal triplet reading frame so that codons downstream from the mutation are out of register and not read properly.

Giemsa banding (G-banding) Method of staining chromosomes that produces light and dark bands characteristic for each chromosome.

gamete Mature reproductive cell (sperm or ovum); contains a haploid set of chromosomes (23 for humans).

gene The fundamental unit of heredity. Functionally defined by its product. Structurally defined as an ordered sequence of nucleotides located in a particular position on a particular chromosome that includes regions involved in regulation of expression and regions that code for a specific functional product.

gene targeting Artificial modification of a gene in a specific and directed fashion. Typically refers to substituting one DNA sequence for another to inactivate a gene or introduce or correct a mutation in a gene.

genetic locus A specific position or location in the genome.

genome The complete genetic information of an organism, usually described as total number of base pairs; human genome contains 3×10^9 base pairs.

genomic DNA DNA from the genome containing all coding (exon) and noncoding (intron and other) sequences, in contrast to cDNA, which contains only coding sequences.

genomic library A collection of clones containing DNA inserts of DNA fragments representing the entire genome of an organism.

genotype The genetic constitution of an individual or organism.

germ cell See gamete.

germ line mosaicism Presence of 2 or more cell lines that differ in genetic makeup among germ cells; implies risk of transmission of mutations present in the gonads to offspring.

gonadal mosaicism See germ line mosaicism.

heterochromatin Chromatin composed of repetitive DNA; stains as dark (vs light) bands in G-banding.

heterozygosity The presence of different alleles at a given genetic locus.

histones Proteins associated with DNA in chromosomes in the nucleus of the cell.

homeobox domain A short DNA sequence common to the genes of many DNA binding proteins.

homeobox (HOX) genes Family of genes conserved throughout evolution that share a common DNA binding domain and encode DNA binding proteins involved in regulation pattern formation during early embryologic development.

homologies Similarities found in DNA or protein sequences of individuals of the same or different species.

homologous chromosomes Chromosomes containing the same linear gene sequences. In a normal mating, 1 of a pair of homologous chromosomes is derived from each parent. Humans normally have 22 pairs of homologous chromosomes and 2 X chromosomes or 1 X and 1 Y chromosome.

homologous recombination Substitution of a segment of DNA by another that is identical (homologous) or nearly so. Occurs naturally during meiotic recombination; also used in the laboratory for gene targeting to modify the sequence of a gene.

homozygosity Presence of the same allele at a given genetic locus.

housekeeping genes Genes that encode proteins necessary for basic cellular functions. They are expressed in virtually all cells.

Human Genome Initiative (Project) Collective name for several projects designed to map and eventually sequence the human genome.

hybridization The artificial pairing of 2 complementary strands of DNA or 1 each of DNA and RNA to form a double-stranded molecule. One strand is often labeled and used as a probe to detect the presence of the second strand.

imprinting Phenomenon in which an allele at a given locus is altered or inactivated depending on whether it is inherited from the mother or father. Implies a functional difference in genes inherited from the 2 parents.

initiation codon The trinucleotide (AUG) that signals the start of translation of a protein.

in situ hybridization Use of a nucleic acid probe to detect the presence of a DNA sequence in chromosome spreads or in interphase nuclei or an RNA sequence in cells. It is used to map gene sequences to chromosomal sites and to detect gene expression.

insert In molecular genetics, refers to a DNA sequence of interest that has been inserted into a cloning vector such as a plasmid or bacteriophage.

insertion Type of mutation in which a DNA sequence of variable length is inserted into a gene, disrupting the normal structure of that gene.

intervening sequences See introns.

introns DNA sequences that interrupt the protein-coding sequences of a gene. They are removed during processing of mRNA. Introns may contain sequences involved in regulating expression of a gene.

kilobase (kb) 1,000 base pairs of DNA sequence.

knockout Term commonly used to describe inactivation of a gene by gene targeting.

library Collection of clones in which genomic DNA or cDNA fragments have been inserted into a cloning vector.

linkage The close proximity of 2 or more genetic loci on a chromosome. The loci can be genes responsible for certain traits or inherited diseases or DNA markers. Closely linked loci are usually inherited together since the closer 2 loci are to each other the lower the likelihood of recombination during meiosis.

locus The position on a chromosome, usually that of a gene, but may refer to a DNA marker.

lod score Literally refers to the log of the odds. A statistical term applied to a set of linkage data to indicate if 2 loci are linked or unlinked. A lod score of +3 (1,000:1 odds) or more is commonly accepted to show linkage and a score of -2 (100:1 odds against) or less excludes linkage.

marker A detectable physical location on a chromosome. It can be a restriction enzyme cutting site, gene, minisatellite, or microsatellite (ie, dinucleotide or trinucleotide repeat or variable number tandem repeat nucleotide) polymorphism whose presence and inheritance can be monitored.

maternal inheritance Inheritance pattern displayed by mitochondrial genes that are propagated from one generation to the next through the mothers; the mitochondria of the zygote come almost entirely from the ovum.

megabase (Mb) One million base pairs of DNA sequence; roughly equal to 1 centimorgan of genetic distance.

meiosis The type of cell division that occurs during gamete formation and results in the halving of the diploid number of chromosomes so that each gamete is haploid and contains 1 of each chromosome pair.

messenger RNA (mRNA) Processed RNA that serves as a template for protein synthesis or for synthesis of cDNA.

methylation See DNA methylation.

microsatellites Highly polymorphic DNA markers comprised of mononucleotides, dinucleotides, trinucleotides, or tetranucleotides that are repeated in tandem arrays and distributed throughout the genome. The best studied are the CA (alternatively GT) dinucleotide repeats. They are used for genetic mapping.

minisatellites Highly polymorphic DNA markers comprised of a variable number of tandem repeats that tend to cluster near the telomeric ends of chromosomes. The repeats often contain a repeat of 10 nucleotides. They are used for genetic mapping.

missense mutation Mutation that causes one amino acid to be substituted for another.

mitochondrial (mt) DNA DNA distinct from nuclear DNA in that it is mostly unique sequence DNA and codes for proteins that reside in mitochondria.

mitosis The type of cell division that occurs in somatic cells in which a cell duplicates itself and its genetic material.

mosaicism Condition in which an individual harbors 2 or more genetically distinct cell lines; results from a genetic change after formation of a zygote, ie, postzygotic event.

motif Three-dimensional structure of gene product (protein) with known or implied function, eg, DNA binding, membrane spanning. A motif is often inferred from cDNA sequence.

mutation A permanent and heritable change in genetic material. Types of mutations include point mutations, deletions, insertions, and changes in number and structure of chromosomes.

nick translation Method used to introduce ^{32}P into a DNA probe so that the probe can be detected.

nonsense mutation Mutation that changes a codon for an amino acid to a termination or stop codon and leads to premature termination of translation.

northern blot Method by which RNA is analyzed. RNA is separated by size, transferred to a membrane (blotted), and detected by a complementary labeled probe that hybridizes to a specific species of RNA, revealing information about its identity, size, and abundance.

nucleosome The basic structural unit of chromatin, in which DNA is wrapped around a core of histone molecules.

nucleotide A purine or pyrimidine base to which a sugar (ribose or deoxyribose) and 1, 2, or 3 phosphate groups are attached.

nucleus The organelle in eukaryotic cells defined by the nuclear membrane that contains the chromosomes.

oligonucleotide A short fragment of single-stranded DNA, typically 5 to 50 nucleotides.

open reading frame (ORF) A sequence of DNA following an initiation codon that does not contain a stop codon. Detection of an open reading frame in DNA implies the presence of a gene that codes for a protein.

ORF See open reading frame.

pax (genes) Paired-box containing genes found in many species that are involved in regulation of early embryogenesis. Pax genes code for (DNA binding) transcription factors. The paired box refers to a particular conserved DNA sequence that is shared by the different members of the pax gene family.

PCR See polymerase chain reaction.

penetrance Refers to clinical expression of a gene or mutation of a gene. If a mutation produces a recognizable phenotype in a patient, the mutation is said to be penetrant. Reduced penetrance means that individuals who harbor a mutation do not always manifest the mutant phenotype clinically.

phage See bacteriophage.

phenotype The appearance (physical, biochemical, and physiologic) of an individual that results from the interaction of environment and genotype. Often used to define the consequences of a particular mutation.

physical map A map of physical landmarks on a DNA fragment or chromosome measured in base pairs. Landmarks include restriction endonuclease recognition sites, DNA sequence, and chromosomal bands.

plasmid Small, circular extrachromosomal DNA molecule capable of autonomous replication within a bacterium. Commonly used as a cloning vector for small pieces of DNA (typically 50 to 5,000 base pairs) by insertion into the plasmid.

poly A RNA RNA transcript that contains a tail of poly A residues at its 3' end; implies that an RNA sequence is mRNA.

polyamines Compounds with many amino groups that are associated in the cell with nucleic acids.

polymerase chain reaction (PCR) A method to amplify a DNA sequence using a heat-stable polymerase and 2 sets of primers that define the sequence to be amplified. Several variations have been developed for specific needs. May be combined with reverse transcription of mRNA to cDNA to amplify an mRNA, so called RT-PCR.

polymorphism The occurrence of 2 or more genetically determined forms. Applied to many situations ranging from genetic traits or disorders in a population to the variation in the sequence of DNA or proteins.

positional cloning Strategy for identifying and cloning a gene based on its location in the genome rather than the biologic function of its product. Usually involves linking the gene locus of interest to one that has already been mapped.

premutation A permanent and heritable change in a gene that does not have phenotypic consequences (does not cause disease) but predisposes to a "full" mutation that may.

primary transcript The initial RNA transcript of a gene, before processing to mRNA; it contains introns as well as exons.

primer Short single-stranded oligonucleotide that anneals to a nucleic acid template and promotes copying of the template starting from the primer site.

proband The propositus (or proposita), or index case, that brings the family to medical attention.

probe Single-stranded DNA or RNA molecule of specific base sequence, labeled either radioactively or by other means, that is used to detect a complementary base sequence by hybridization.

promoter A sequence on a gene that is upstream (5') to coding sequences to which RNA polymerase binds and initiates transcription of the gene.

pseudogene Sequence of DNA that is very similar to a normal gene but has been altered slightly so that it is not expressed.

reading frame Register in which translation machinery reads the genetic triplicate code.

recessive mutations Mutations that produce an abnormal clinical phenotype when present in the homozygous or hemizygous state. Heterozygosity for the mutation, ie, carrier state, may often be detected in persons whose clinical phenotype is normal.

recombinant DNA molecules DNA molecules of different origins that are combined and manipulated in the laboratory.

recombinant DNA technologies Laboratory procedures used to manipulate DNA fragments, eg, cut, modify, ligate, etc, and introduce them into an organism so that their number can be amplified as the organism replicates, ie, cloning.

recombination The formation of new combinations of linked genes by crossing over between their loci during meiosis.

restriction enzyme, restriction endonuclease Bacterial-derived enzyme that recognizes specific, short nucleotide sequences and cuts DNA at that site.

restriction fragments DNA fragments that result from digestion of DNA with restriction enzymes.

restriction fragment length polymorphism (RFLP) Genetic variation resulting from a difference in DNA sequence that affects the recognition sequence for restriction enzymes. When DNA is digested by a particular enzyme, the fragment sizes will differ, depending on the presence or absence of the proper recognition sequence for the enzyme.

restriction map A map of a DNA sequence with restriction enzyme recognition sites serving as landmarks.

restriction site Shortened term for restriction endonuclease recognition sequence.

retroviruses RNA viruses that encode the enzyme reverse transcriptase so that their RNA can be transcribed into DNA in the host cell; modified retroviruses are used as vectors to introduce genes (or portions thereof) of interest into eukaryotic cells.

reverse transcriptase An enzyme that catalyzes the synthesis of DNA from an RNA template.

RFLP See restriction fragment length polymorphism.

RNA polymerase Enzyme that synthesizes (transcribes) RNA from a DNA template.

RNA splicing Process by which introns are removed from primary RNA transcripts, leaving only exons that encode the amino acid sequence of a protein.

sequence Order of bases in DNA or RNA or of amino acids in a protein.

sequence-tagged sites (STSs) Short sequences of genomic DNA for which the base sequence is known. Polymerase chain reaction can be used to amplify the known sequences, which can serve as physical landmarks for mapping.

sequencing Determination of the order of nucleotides in a DNA or RNA fragment, or the order of amino acids in a protein.

sequencing gel analysis Electrophoretic technique by which nucleotide size differences as little as a single base pair can be discerned.

somatic cell hybrid A hybrid cell line derived from fusion of cells from different sources. Human/rodent hybrids containing a small amount of human genetic material, such as a single chromosome, are used in human gene mapping.

somatic cells All cells in the body except gametes and their precursors.

somatic mosaicism Presence of 2 or more cell lines that differ genetically in somatic (non-germ line) cells.

Southern blot (hybridization) Method by which DNA is analyzed that was originally described by E. M. Southern. DNA is fractionated by electrophoresis, transferred to a membrane (blotted), and detected by a complementary labeled probe that hybridizes to the DNA, revealing information about its identity, size, and abundance.

splicing See RNA splicing.

STSs See sequence-tagged sites.

synteny Refers to the presence of 2 or more loci on the same chromosome; they may or may not be linked closely. For example, 2 gene loci that map to the distal and proximal locations on the long arm of chromosome 1, respectively, would not be linked but would exhibit synteny.

tandem repeat sequences Multiple copies of the same base sequence on a chromosome. When the number of repeats varies in the population, they are useful as DNA markers.

TATA box A conserved sequence 25 to 30 base pairs upstream from the start site of transcription, in many but not all genes; binding site for general factors involved in initiation of transcription.

telomeres Refers to the ends of chromosomes that contain characteristic repetitive DNA sequences.

termination (or stop) codon One of the 3 codons (UAG, UAA, or UGA) that causes termination of protein synthesis.

trans (1) Historically implies on a different chromosome. (2) In molecular biology, refers to an effect on a gene caused by a factor distinct from the sequence of that gene, in contrast to cis effects, which are encoded in the sequence of the gene. Cis and trans are commonly used to describe factors that influence gene expression.

transfection Transfer of a DNA fragment into prokaryotic or eukaryotic cells.

transcript Refers to an mRNA molecule that encodes a protein.

transcription The synthesis of an RNA molecule (transcript) from a DNA template in the cell nucleus catalyzed by RNA polymerase.

transcription start site Site within a gene where transcription of RNA begins.

transgenic Containing foreign DNA, eg, transgenic mice contain foreign DNA sequences in addition to the complete mouse genome.

translation Assembly of amino acids into peptides based on information encoded in mRNA, ie, mRNA sequence of

bases is translated into sequence of amino acids in a peptide or protein. Occurs on ribosomes.

translocation The exchange of genetic material from one chromosome to another nonhomologous chromosome, usually through a reciprocal event at meiosis.

uniparental disomy Situation in which an individual has 2 homologous chromosomes (homologues) or chromosomal segments from 1 parent and none from the other. May be heterodisomy if 1 copy of each of the homologues from the single parent are present or isodisomy if 2 copies of the same homologue are present.

unique sequence DNA Nonrepetitive DNA that potentially codes for mRNA and protein.

upstream A DNA sequence is written from the left, or 5', direction or to the right, or 3', direction. Upstream refers to the 5' direction, ie, regulatory elements of a gene are typically located upstream (5') of the coding sequences of that gene.

variable number tandem repeat (VNTR) A type of DNA marker. See marker.

vector See cloning vector.

western blot Method by which proteins are analyzed. Terminology based on convention of Southern (DNA) and northern (RNA) blots. Proteins are fractionated by electrophoresis, transferred to a membrane (blotted), and detected by a labeled probe, usually an antibody. Provides information about size, abundance, and identity of the protein.

X-inactivation The inactivation of most of the genes on 1 of the X chromosomes in female somatic cells during early embryonic development.

YAC See yeast artificial chromosome.

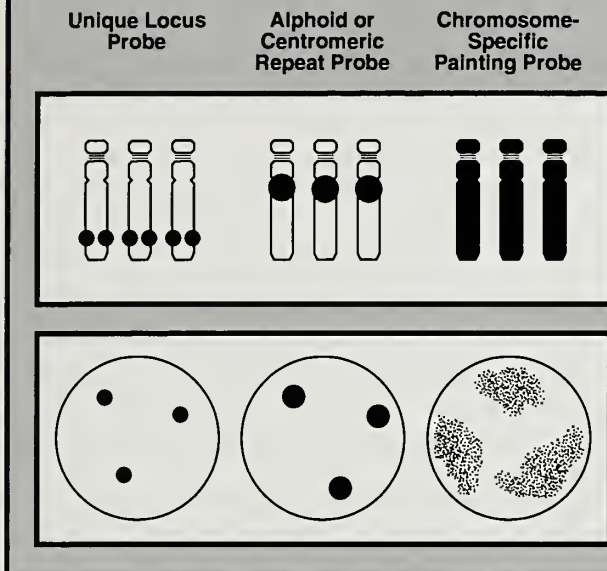
yeast artificial chromosome (YAC) A vector used to clone large DNA fragments. The inserts can be much larger than those accepted by other vectors, such as plasmids or cosmids.

zygote The diploid cell resulting from the union of the haploid male (sperm) and female (ovum) gametes.

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Figure 2
Diagrammatic Representation of Trisomy 21 Detection by FISH



(A) The hypothetical appearance of chromosome 21 in a metaphase spread when hybridized with each of the appropriate probe types: Black indicates probe-specific fluorescent signal. Thus, a locus-specific probe gives a sharp, discrete signal at its relevant position. An alphoid or centromeric repeat probe gives a large, more diffuse signal near the centromere. A painting probe decorates the entire chromosome. The copy number of the specific chromosome can be determined with any of the probe types.

(B) The appearance of G1 interphase nuclei after hybridization with each of the probe types: The locus-specific probe gives the best resolution and also would detect duplication of the Down syndrome critical region as a result of translocation. The alphoid repeat probe gives a bright and discrete signal but would not necessarily detect a translocation. Because of the diffuse nature of the chromosomal domain in interphase, overlapping domains visualized with the painting probe can make chromosome enumeration more difficult.

CLINICAL APPLICATIONS

Clinical applications of FISH technology take advantage of molecular probes that are specific for defined regions of cytogenetic interest. The methodology widens the scope of what is diagnosable by the clinical cytogenetics laboratory because it is based on microscopic visualization to determine the copy number of specific DNA sequences in the target. Since the technique permits visualization of changes at a sensitivity beyond what can be seen on banded chromosomes with the light microscope, many additional cases of constitutional cytogenetic abnormality are identifiable, and appropriate recurrence risks and prognostic information for the associated disorders can be effectively established for the family (Table 2).¹ Specific karyotypic abnormalities for which FISH is useful are discussed below.

Aneuploidy: An example of the use of FISH to document autosomal trisomy is diagrammatically represented in Figure 2. A hypothetical unique locus probe, specific for the Down syndrome critical region on the distal long arm of chromosome 21, has been used as an example for detecting trisomy 21 by hybridization to metaphase chromosomes as shown in Panel A or to

interphase nuclei as shown in Panel B. As is shown in the remainder of Figure 2, similar studies could be accomplished with the use of an alphoid probe specific for the centromeric region of the chromosome, or by painting the entire chromosome with a chromosome 21 painting cocktail. These studies also can be effectively used to rapidly assess the presence of all clinically significant trisomic conditions during prenatal diagnosis² or during the newborn period, and when questions of clinical management require timely information regarding the karyotype of the fetus or the neonate. In addition, one can envision numerous applications using interphase nuclei to assess tumor specimens for ploidy or for additional copies of a specific chromosome. An interphase nucleus from a pediatric glioma hybridized with a chromosome 7 alphoid sequence that demonstrated 3 copies of chromosome 7 is shown in Figure 1D. Extra copies of chromosome 7 have been reported in adult gliomas, but trisomy 7 was not demonstrable in metaphase preparations from the pediatric tumor presented in Figure 2. Thus, FISH may be more sensitive than standard cytogenetic analysis for detection of aneuploidy in tumors.

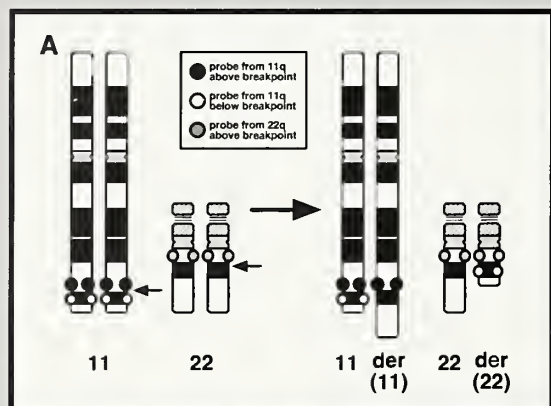
Translocations: The identification of nonrandom translocations in neoplastic cells using FISH is a rapidly expanding field. The molecular description of these tumor-associated translocations has enabled the development of FISH-based assays for clinical evaluation of the appropriate leukemias, lymphomas, and solid tumors. Although cytogenetic analysis is unmatched in its ability to define the full constellation of chromosomal changes in a tumor, sequence-based assays utilizing FISH offer several advantages for detection of specific chromosomal rearrangements. These advantages include greater sensitivity, decreased time and cost per assay, small sample size, and obviation of cell culture.

Table 2
FISH Technology

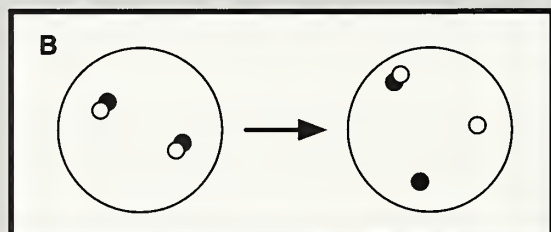
Clinical Applications

- Prenatal diagnosis of cytogenetic abnormality
- Assessment and management of the critically ill newborn with suspected aneuploidy
- Tumor karyotype identification at initial diagnosis
- Assessment of minimal disease

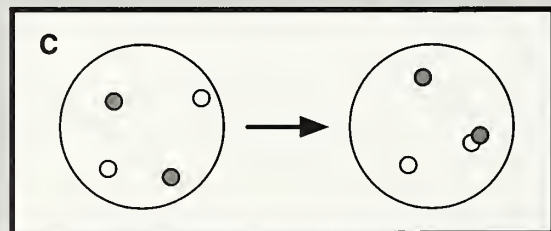
Figure 3
Diagrammatic Representation of Ewing's Sarcoma t(11;22) Translocation Breakpoint Detection by FISH



(A) An ideogrammatic representation of the t(11;22) showing the location of 3 single copy probes that could be used to detect the translocation: The small arrows to the right of the chromosomes indicate the position of the translocation breakpoints on chromosomes 11 and 22. Probes are used in pair-wise combinations. Either a pair of probes that flank the breakpoint on 1 of the 2 involved chromosomes (for example ● and ○ on chromosome 11) or a pair of probes that are below the breakpoint on 1 of the involved chromosomes and above the breakpoint on the other (for example the ○ probe from chromosome 11 and the ● probe chromosome 22).



(B) Diagrammatic representation of results of FISH with breakpoint-flanking probes from chromosome 11 on normal (left) and t(11;22)-positive Ewing's sarcoma interphase nuclei (right): The fluorescent signals become separated in the interphase nucleus as a result of the translocation.



(C) Diagrammatic representation of results of FISH with translocation breakpoint-related probes (for example ○ from below the breakpoint on chromosome 11 and ● from above the breakpoint on chromosome 22): The chromosome 11 and chromosome 22 probe signals that are normally separated in interphase (left) are brought into juxtaposition (right) as a result of the translocation.

For example, an application of FISH technology for t(9;22) rearrangement detection in interphase nuclei has been described. Simultaneous hybridization of differentially labeled probes for *bcr* and *abl* to chronic myelogenous leukemia (CML) bone marrow nuclei detects co-localization of the 2 genes as a result of the translocation.³ Similar studies have been accomplished for the t(11;22) translocation of Ewing's sarcoma, a solid tumor that is difficult to diagnose.^{4,5} The assays for such tumor rearrangements use either a pair of probes derived from one of the translocation partners, which co-localize in normal cell nuclei and are distinctly separated in tumor nuclei as a result of the translocation, or, alternatively, a pair of probes from both translocation partners, which are separated in normal nuclei and co-localize in tumor nuclei as a result of the translocation. This is shown diagrammatically in Figure 3.

Once a suitable pair of probes is identified, FISH of interphase nuclei from tumors can be utilized as a rapid assay for the translocation. This assay should eventually have applicability to a variety of clinical material. Since this assay can be accomplished much more quickly than the standard cytogenetic analysis, FISH analysis of biopsy material will be clinically advantageous in difficult diagnostic situations.

Microdeletion Syndromes: One of the most challenging tasks in human clinical cytogenetics is the identification of cytogenetically undetectable microdeletions in association with phenotypic features of known deletion syndromes. Several examples are: Wolf-Hirschhorn (4p-), cri du chat (5p-), Langer-Gideon (8q-), Prader-Willi and Angelman (15q-), Miller-Dieker (17p-), Alagille (20p-), and DiGeorge and velocardiofacial (22q-) syndromes. Analysis of small deletions of human chromosomes is best approached by hybridizing with probes that are targeted to the critical deletion region that is responsible for the syndrome. An example of such an approach for velocardiofacial syndrome is shown photographically in Figure 1E (page 8) and diagrammatically in Figure 4. In the photograph, a cosmid for the DGS critical deletion region has been hybridized to metaphase chromosomes from a possible DGS patient. The metaphase chromosome demonstrates the microdeletion as absence of hybridization to 1 of the chromosome 22 homologues. This is shown diagrammatically in Figure 4, which also demonstrates that the interphase nucleus would show a single signal.

Marker Chromosomes: Small additional marker chromosomes in the human karyotype are impossible to identify because of their paucity of banding landmarks. Numerous researchers have recently taken advantage of the centromere-specific alphoid satellite probes to determine the origin of such marker

chromosomes.⁶ This technique provides information regarding the origin of the marker chromosome, which has significance for patient management. In addition, numerous patients with sex chromosomal abnormalities have a karyotype characterized by the presence of a single X chromosome while the second sex chromosome is replaced by a small marker chromosome, presumed to be derived from the missing sex chromosome. Identification of the origin of the small marker chromosome is easily accomplished by FISH using probes for the centromeric region of the X and Y chromosomes.

Sex Chromosomal Assessment: Probes that identify the centromeric regions of the X and Y chromosomes also are useful for monitoring patients who have had sex-mismatched bone marrow transplants. With FISH one can easily identify residual host cells in marrow aspirate by their sex chromosome content. In this instance, interphase cytogenetics may prove to be extremely useful during therapy, when there is a paucity of cells available on which to perform standard cytogenetic analysis. Sex chromosome identification also may prove useful in prenatal diagnosis of X-linked disease.

Gene Amplification: Oncogene amplification is associated with a poor prognosis for several tumors. This is particularly true for *N-myc* amplification in neuroblastoma and *erb B2* amplification in breast tumors.⁷ These amplified sequences can be readily detected in meta-

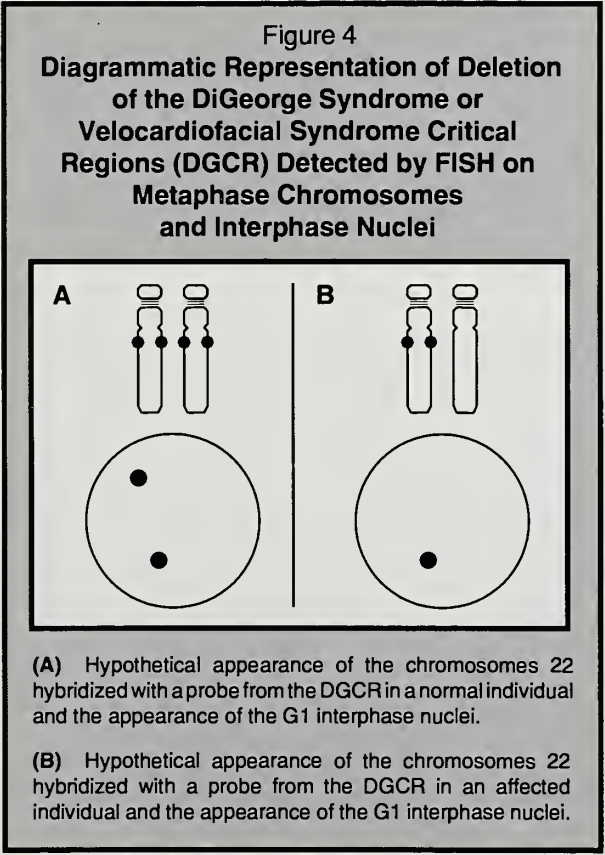
phase and interphase using FISH. Probes for these markers are commercially available for use in tumor staging and management.

De Novo Additions: The de novo addition of unidentifiable chromosomal material to a recognizable human chromosome represents a dilemma for the cytogeneticist. Such material is often identified in a dysmorphic newborn whose parents' karyotypes are normal; questions are then raised regarding prognosis and management based on the identification of the additional material. The results of such a study are seen in Figure 1F (page 8). In this patient, de novo additional material on the short arm of one chromosome 15 was suspected of being derived from chromosome 17. FISH with cosmid probes designed to detect the Miller-Dieker deletion on 17p (ONCOR; Gaithersburg, Md) was successful in allowing identification of the origin of the extra chromosomal materials.⁸ In addition, numerous tumor specimens contain chromosomes with additional material that is difficult to identify. It would be useful to determine the composition of such aberrant chromosomes. In many cases, the approach is chromosome-specific probe hybridization or chromosome painting using locus-specific or composite paint probes judged to be the most likely candidates from the banding pattern. The other approach to such a situation, although not as readily available, would be microdissection followed by PCR and hybridization of the amplified microdissected material back to normal human metaphase chromosomes. This is a very powerful technique, one probably best handled in a limited number of reference laboratories rather than in typical diagnostic cytogenetic laboratories.

In summary, the genetic and oncologic diagnostic applications of FISH are increasing steadily with the availability of chromosome- and disease-specific reagents. The parallel development of sophisticated microscopy and detection systems, in addition to their greater accessibility, is enhancing the capability of routine clinical diagnostic laboratories to perform such analyses, allowing for easier and more accurate assessment of specimens. The technology has enormous potential and critical implications for the future of cytogenetics. With FISH, the enumeration and localization of the DNA sequences underlying a cytogenetic abnormality can be accomplished by direct visualization at the microscope with both sensitivity and accuracy.

ACKNOWLEDGEMENT

The author wishes to acknowledge Drs. Jaclyn Biegel and Nancy Spinner for materials reproduced in this paper and for productive discussions. The technical assistance of Beatrice Sellinger, Annette Parmiter, and Nancy Owens also is gratefully acknowledged. The FISH studies reproduced in this manuscript were supported in part by funds from CA39926 and CA46274 from the National Institutes of Health.



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Abstracts From the Literature

Comparative Assessment of Dual-Photon Absorptiometry and Dual-Energy Radiography

Dual-energy bone densitometry can be performed with 2 types of scanners, dual-photon absorptiometry (DPA) and dual-energy radiography (DER). DPA uses an isotope source (gadolinium-153; ¹⁵³Gd) and was developed in the 1960s and 1970s. DER, which was developed in the late 1980s, uses an incorporated X-ray tube. DER also is known as DRA (dual-energy radiographic absorptiometry), QDR (quantitative digital radiography), and DEXA (dual-energy X-ray absorptiometry). Glüer et al, authors of this paper, suggest that DER be used exclusively.

Comparison of the basic principles, advantages, and disadvantages of DPA and DER, as presented in the current paper, are tabulated in the accompanying table. Comparison was made for both bone mineral density (BMD) and bone mineral content (BMC). Normal healthy adults and females with and without osteoporosis were studied.

The authors discuss that the underlying concepts of DPA and DER are very similar in that they both are based on dual-energy projection (3-dimensional) scanning. Development of DER relates to improvements in technology, particularly to the enhancement of X-ray beam intensity by replacement of the ¹⁵³Gd isotope source with an X-ray tube and, in some machines, the incorporation of internal calibration devices. The authors state that comparative assessment of the 2 densitometers demonstrates that these technologic improvements have resulted in marked progress in a number of clinically important performance characteristics. For example, the results reveal a significant improvement in precision with DER. The marked enhanced resolution accounts for the significantly increased precision. This improvement was found to

be of particular importance for the analysis of spinal BMD.

In clinical practice, the scanning time of 6 to 7 minutes for DER, as compared with 20 to 45 minutes for DPA, represents important progress. Errors caused by patient movement are reduced, better utilization of the equipment is achieved, and, therefore, cost is reduced.

In conclusion, the precision, spatial resolution, and scanning time of DER are significant improvements over those of DPA. Fortunately, normative data obtained by using DPA can be used for DER studies. The bone mineral values for individual patients can be corrected by the average differences between DER and DPA.

Glüer CC, Steiger P, Selvidge R, et al. *Radiology* 1990;174: 223-228.

Editor's comment: Although this article was published in 1990, its importance to those who are becoming involved in measurements of BMD and BMC prompts our abstracting it for GGH.

This article can serve as a foundation for those who have an interest in extending their knowledge of DPA and DER. Finally, the improvements to DER permit changes in BMD and BMC to be detected over a period of months instead of years, when therapeutic agents are tested.

Robert M. Blizzard, MD

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	Dual-Photon Absorptiometry (DPA)	Dual-Energy Radiography (DER)
Basic Principles	1. ¹⁵³ Gd isotope source 2. Photon-counting detector 3. Multiple accessible bone sites	1. X-ray tube source 2. Alternating X-ray generator voltage with integrating detector
Advantages	1. Low X-ray dose 2. Clinically adequate accuracy	1. Reduced scanning time (6-7 min) ...patient comfort, equipment utilization 2. Improved resolution ...reduced precision error
Disadvantages	1. Long scanning time (20-45 min) 2. Limitations in precision (up to 6%)	

Magnetic Resonance Imaging in the Diagnosis of Growth Hormone Deficiency

In this study, 46 patients (29 male) with idiopathic growth hormone (GH) deficiency were examined by magnetic resonance imaging (MRI) at a mean (\pm SEM) age of 9 ± 1 years (range, 15 days to 20 years). At the time of evaluation, 37 patients were prepubertal, 5 had spontaneous pubertal development, and 4 were receiving supplementary testosterone or estrogen-progesterone therapy. MR images were obtained before therapy with human GH (hGH) ($n=28$), during hGH therapy ($n=13$), or after hGH therapy ($n=5$). The diagnosis was confirmed by a GH peak response <8 ng/mL after 2 pharmacologic stimulation tests.

In all cases, T1-weighted images, 3 mm thick, were obtained in the sagittal and coronal planes. The maximal height of the pituitary gland was measured in a plane perpendicular to the floor of the sella turcica. Ischemic lesions of the hypothalamus and basal ganglia were looked for on 5 mm thick T2-weighted coronal images.

The patients were classified into 2 groups according to MR images: group 1 ($n=29$) had pituitary stalk interruption syndrome (PSIS) and group 2 ($n=17$) had normal pituitary anatomy. PSIS was diagnosed based on the following criteria: lack of visible pituitary stalk, lack of the normal posterior lobe hypersignal into the sella turcica, and presence of a hyperintense nodule in the region of the infundibular recess of the third ventricle. All patients with PSIS had a pituitary height <2 standard deviations (SD) for age; 3 had no visible anterior pituitary lobe. The pituitary height was less than normal in 10 patients (60%) with normal pituitary anatomy.

The group with PSIS had the first symptom of GH deficiency at an earlier age (2.8 ± 0.6 years vs 5.5 ± 1.2 years; $P<0.001$), were of smaller stature (-4 ± 0.2 SD vs -3 ± 0.2 SD; $P<0.01$), and had lower GH peak response to provocative testing (3 ± 0.4 ng/mL vs 5 ± 0.5 ng/mL; $P<0.001$) than the group with normal pituitary anatomy. Their pituitary gland also was shorter (2.5 ± 0.2 mm vs 3.5 ± 0.2 mm; $P<0.01$). All the patients with multiple pituitary deficiencies except 1 ($n=19$) belonged to this group. There was a higher incidence of multiple pituitary deficiencies (65% vs 5%) in the PSIS group ($P<0.001$). There also were more perinatal abnormalities (24% vs 17%), associated congenital abnormalities (17% vs 12%), microphallus (15% vs 11%), and hypoglycemia (14% vs 0%) in the PSIS group compared with the group with normal pituitary as determined by MRI.

The authors concluded that the evaluation of the shape and height of the pituitary gland by MRI is an additional tool for the diagnosis of PSIS-related GH deficiency. The presence of pituitary stalk interruption confirms this diagnosis and is predictive of multiple anterior pituitary deficiencies.

Argyropoulou M, Perignon F, Brauner R, et al. *J Pediatr* 1992;120:886-891.

Editor's comment: This study clearly demonstrates that MRI is of value (when performed using the appropriate equipment and personnel) for the evaluation of patients with GH deficiency. MRI studies in hypopituitarism often add information to confirm the diagnosis. The authors showed that MRI is the best clinical tool to document PSIS, which cannot be diagnosed with certainty by any other clinical or radiologic methods. MRI may even contribute data regarding the duration and long-term prognosis of the pituitary deficiencies.

A decreased pituitary height and/or the presence of pituitary stalk interruption as demonstrated by MRI will assist in the interpretation of the biochemical and hormonal data. For example, MRI abnormalities would help elucidate the depressed, spontaneous,

or stimulated GH levels in an obese short child. It also may help the clinician in interpreting the GH levels utilized as a cutoff level for diagnosis of GH deficiency. The demonstration of MRI abnormalities of the pituitary stalk also should alert the clinician to the possibility of multiple hormonal deficits.

However, MRI findings do not necessarily correlate with pituitary function.¹ In one study, the height of the anterior lobe of the pituitary gland in 17 of 22 children with idiopathic GH deficiency was less than 3 mm.² Although anatomic abnormalities of the hypothalamic-pituitary axis are more commonly observed in patients with multiple pituitary defects, there is overlap between these patients and those with isolated GH deficiency.¹ Thus, MRI cannot obviate a complete endocrine workup. The authors of this study did not comment on the presence of ectopic pituitaries as a possible distinct entity from PSIS. The authors did not state in what number of patients MRI demonstrated the presence of a hyperintense nodule in the third ventricle, with or without evidence of pituitary stalk alterations. Evidence suggestive of an "ectopic" posterior pituitary gland in children with idiopathic anterior hypopituitarism was shown in previous MRI studies.³ This, and numerous other studies, confirms that MRI is expanding the capabilities of endocrinologists to interpret suspected pathophysiology.

Fima Lifshitz, MD

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Erratum

In *GROWTH, Genetics, & Hormones* Vol. 8, No. 4 (December 1992) an error on page 7 under the section entitled Sleep Staging, (the first sentence) incorrectly identifies "(stages 1 to 3)". This should have been provided as *references* 1 to 3.

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Growth Hormone and Tumour Recurrence

Ogilvy-Stuart et al report data on the recurrence of CNS tumors in children in the northwest region of England who were treated with human growth hormone (GH). Included in the analysis were 207 children with brain tumors between 0.5 and 14.4 years of age (median, 6.7 years); 47 of these (29 boys) received GH. The median length of time from diagnosis to initiation of therapy was 4.5 years, and the median duration of therapy was 3.2 years. Serving as a comparison group were 160 children who had not been treated with GH. Each child had received cranial irradiation (median dose, 3000 cGy); 36 children had received a tumor-site booster dose (median dose, 1500 cGy). All were evaluated for GH deficiency at approximately 2 years postradiotherapy, a time when tumor recurrence is most likely to occur and also a time at which tumor-induced GH deficiency may be readily identified. The dose of GH was 12 IU/wk prior to 1989 and 0.5 IU/kg/wk after 1989.

Five of the 47 children (11%) who were treated with GH had a clinical relapse associated with recurrence of brain tumor. In 2, relapse occurred after the completion of the GH treatment, while in 3 patients relapse occurred from 0.5 to 3.3 years after starting therapy. Forty-two of 160 children (26%) who did not receive GH

relapsed. Thus, the authors conclude that there is no association between GH and tumor recurrence.

Ogilvy-Stuart AL, Ryder WDJ, Gattamaneni HR, et al. *Br Med J* 1992;304:1601-1605.

Editor's comment: This is another reassuring study for pediatric endocrinologists assessing children who have received cranial irradiation and who have growth retardation. It is noteworthy that 10 of 44 children with brain tumors who had computed tomography scans performed at the beginning of GH therapy showed residual tumor. Thus, there does not appear to be a clear association between tumor growth and GH treatment. The authors point out that as more children are successfully treated for CNS malignancy, more of these children will be presenting to pediatric endocrinology clinics for possible GH therapy. It is important that similar registry data be continued to ensure that children treated with GH do not show an increased risk of tumor recurrence.

William L. Clarke, MD

Elevated Growth Hormone Secretory Rate in Premature Infants

Wright et al studied 5 premature infants (gestational age, 24 to 34 weeks) and 6 full-term infants for growth hormone (GH) secretory characteristics by drawing blood every 15 minutes for 6 hours for determination of GH concentrations. Deconvolution analyses were done to study the GH secretory characteristics in both groups.

The authors confirmed their own previous work that: premature infants have higher GH concentrations than full-term infants ($18,100 \pm 800 \mu\text{g/L}$ vs $10,200 \pm 2700 \mu\text{g/L}$; $P=0.067$); the half-life of circulating GH for both groups was similar to that reported for normal adult men (18.9 minutes); and premature infants had significantly higher secretory burst amplitudes than full-term infants, as well as higher production rates. The insulin-like growth factor 1 (IGF-1) values were lower in premature infants than in full-term infants.

When these data are interpreted in conjunction with other known data, eg, premature infants have lower levels of IGF-binding protein 3 and GH-binding protein than full-term infants, the authors conclude that the increased GH secretory activity in premature infants reflects an increase in hypothalamic GH-releasing hormone activity and/or reduced somatostatin tone.

Wright NM, Northington FJ, Miller JD, et al. Elevated growth hormone secretory rate in premature infants: deconvolution analysis of pulsatile growth hormone secretion in the neonate. *Pediatr Res* 1992; 32:286-290.

Editor's comment: The authors are to be commended for performing a tedious task and deriving valuable data while drawing only 2.8 mL of blood. The findings provide further understanding of the pulsatile characteristics of GH secretion at a relative early gestational age (24 to 34 weeks).

One must realize, however, that the characteristics of GH secretion are probably not related to fetal growth, as GH is not required for normal or near-normal fetal growth. The factors stimulating fetal growth are probably multiple (see GGH, 8[1]:1), but probably do not include GH, human chorionic somatomammotropin, prolactin, or IGF-1. Regardless, the data reported by Wright et al are valuable for the reasons stated above.

Robert M. Blizzard, MD

The Birth Injury Theory of "Idiopathic" Growth Hormone Deficiency

At the beginning of his article, Dr. Itsuro Hibi gives an historical survey of the origin of the birth injury theory in respect to the etiopathogenesis of idiopathic growth hormone deficiency (GHD). The pathologist Simmonds (1919) was the first to describe a severe atrophy of the adenohypophysis, probably due to birth trauma, in a 21-year-old sexually infantile dwarf. Prader (1960) reported on the birth histories of 25 children with idiopathic GHD. Complicated births were ascertained in 18 cases. In 1962, both Bierich and Van der Werff ten Bosch reported that two thirds of their patients with idiopathic GHD were born in noncephalic positions, mainly by breech deliveries. It has been known for a long time that such births are connected with high mortality and morbidity. In the majority of Bierich's patients (1965), perinatal asphyxias and convulsions also were recorded. In the years

following, these findings were confirmed by numerous authors, including Hibi and Tanae (1979).

Dr. Hibi has considered the following questions: Does GHD result from or cause breech delivery? If GHD can be caused by breech delivery, why is spinal cord injury not found more frequently? If breech delivery causes GHD, why are only a small proportion of children who are born by breech delivery affected by hypopituitarism? How can male preponderance of GHD be explained in relation to the association of breech delivery with GHD?

After studying 95 siblings of 70 idiopathic GHD children born by breech delivery and finding that none of the 20 siblings also born by breech delivery had idiopathic GHD, Dr. Hibi concluded that idiopathic GHD in these 70 patients resulted from the breech delivery.

The author states that there are reports associating spinal cord injury and GHD, and concludes that this supports the thesis that GHD results from breech delivery. However, he readily admits that most patients with GHD and born by breech delivery do not have spinal cord problems.

The author only briefly considers why such a small portion of children born by breech delivery have GHD, and provides no satisfactory answer to this question.

Most intriguing in this paper are the data pertaining to the sex ratio. From these reports concerning 523 patients with idiopathic GHD, the following are retabulated.

Delivery	No. Patients	Male:Female Ratio
Breech	316	5.1 - 7.8*
Vertex	207	1.1 - 1.7*

* According to various series.

The author concludes that the reason why more male than female infants with GHD are born by breech delivery is unclear.

In closing, Dr. Hibi notes that the percentage of patients born by breech delivery among GHD patients in Japan between 1986 and 1988 is very low (6% of 6,357 GHD patients). Of course, mild GHD is much more frequently diagnosed than previously, and it may be that only severe GHD is found in association with breech delivery.

Hibi I. *Clin Pediatr Endocrinol* 1992;(1):1-3.

Editor's comment: Dr. Hibi's article is the first article in a new journal, *Clinical Pediatric Endocrinology*. This is the official English journal of the Japanese Society for Pediatric Endocrinology, and it will be published twice yearly.

Because Professor Jürgen Bierich published extensively regarding this topic, I have asked him to comment. We are proud to note that he is a former Editorial Board member of GGH. His reply is as follows:

Hibi and Tanae (1979) investigated 95 siblings of 70 breech-born patients with idiopathic GHD. Twenty of these 95 also were born by breech delivery. Therefore, the author drew the conclusion that the GHD of the initial 70 patients was the result of the breech delivery—an event that leads to GHD in only a minority of cases. The question why only a small fraction of the breech-born children acquire hypopituitarism remains unanswered in Dr. Hibi's paper. In my opinion, the reason lies in the fact that breech deliveries exhibit rather variable courses. Usually, but by no means in all of the cases, they are connected with additional complications, eg, prematurity, prolapse of the umbilical cord, and early abruption of the placenta. Whether hypothalamic injury occurs depends on the obstetric situation in toto.

To seriously consider the pathophysiologic basis of GHD possibly associated with breech delivery, one must consider the anatomy that has been found in association with isolated GHD. The few autopsy records of patients with idiopathic GHD have shown severe atrophy of the adenohypophysis with loss of the chromophilic cells. During the last 15 years, several investigations with modern imaging techniques were performed in patients with idiopathic GHD; these showed rather small pituitary glands with no demonstrable stalks. Dr. Hibi concludes that transections of the stalk, or ischemic alterations of the pituitary, represent the typical morphologic correlate of idiopathic GHD. However, older investigations favor the assumption that vaginal breech deliveries cause predominantly cerebral hemorrhages. During the last 15 years, numerous endocrinologic studies have demonstrated that idiopathic GHD in the

majority of cases represents a hypothalamic disorder and rests primarily upon a growth hormone-releasing hormone deficiency. The atrophy of the pituitary is a secondary phenomenon resulting from inadequate central stimulation. Actual spinal cord lesions, in particular syringomyelias, have been observed in patients with idiopathic GHD (Fujita et al, 1992). However, many of these escape the pediatrician's detection because they manifest themselves relatively late, eg, in the second decade of life. It is possible that the incidence of spinal cord lesions is increased in children with idiopathic GHD in association with breech delivery, but the tabulations to evaluate the association have not been determined because the studies were done early in life.

Dr. Hibi states that the reason why more male than female infants with idiopathic GHD are born by breech delivery is unclear. The author discusses the so-called male disadvantage, ie, the generally enhanced susceptibility of male infants to perinatal damage. With regard to mortality, the male:female ratio in the large series of Naye et al (1971) was 1.7:1. For cerebral birth trauma, Prader (1960) found a sex ratio of 2:1 in the children of the Kinderspital Zurich. I am in accordance with Hibi that the male disadvantage can explain the slight preponderance of the male sex in children born in vertex position, but not the extraordinarily high ratio (ranging from 5.1 to 7.8) in the breech-born infants. In order to arrive at a plausible interpretation of these findings, 2 questions require clarification: first, whether breech presentations occur more frequently in male than in female infants; and second, whether breech deliveries are more likely to cause cerebral lesions in boys than in girls. The follow-up study of Manzke (1978) speaks to this question. Among breech-born infants who were reinvestigated at age 6, the male patients exhibited worse neurologic and mental test results than the female ones. The differences were statistically significant, but small. Certainly, investigations of larger cohorts are necessary in order to come to definite answers.

Thank you for inviting my comments.

Jürgen Bierich, MD

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2nd Editor's comment: Although breech delivery occurs more frequently with male children with idiopathic GHD than with female children with idiopathic GHD or than with male children without idiopathic GHD, the answers to Dr. Hibi's questions remain elusive. We thank Dr. Bierich for providing his insight into the problem. He also believes the questions still remain unanswered, and suggests what must be done to supply answers to the questions—if possible.

Robert M. Blizzard, MD

MEETINGS CALENDAR

April 15-18, 1993 Intl Immunol and Diabetes Wkshp (IDW), Orlando, FL. Info: Dr NK Maclaren, Dept of Pathol, Univ of FL, Box 100275, JHMH, Gainesville, FL 32610. Tel: 904-392-6840; Fax: 904-392-6249.

May 3-6, 1993 Amer Pediatr Soc/Soc for Pediatr Research/Ambulatory Pediatr Assoc, Washington, DC. Info: Elk Grove Village, IL. Tel: (SPR) 708-427-0205, (APS) 708-427-1205; Fax: (both) 708-427-1305.

May 26-29, 1993 Wkshp on Non-Conventional GH Therapy - ISGD Course in Therapeutic Aspects of Childhood Diabetes, Siena, Italy. Info: Dr F Chiarelli, Dept of Paediatr, Univ of Chieti, 11 Via Nicolini, 66100 Chieti, Italy. Tel: 39-871-412-72; Fax: 39-871-63-669.

May 27-June 1, 1993 7th Intl Clin Genet Sem on "Dysmorphology" and "Genetics of Cardiovascular Disorders" in Samos, Greece. Info: Dr C Bartsocas, Dept of Paediatr, "P & A Kyriakou" Children's Hosp, GR-11527 Athens, Greece. Tel: +30-1-7709316; Fax: +30-1-7796461.

May 30-June 3, 1993 Amer Soc for Biochem and Molecular Biol, San Diego, CA. Info: M Sternburg, 9650 Rockville Pike, Bethesda, MD 20814. Tel: 301-530-7010; Fax: 301-550-7014.

June 1-2, 1993 Symp on "Male Sexual Differentiation." Info: Dr CJ Migeon, CMSC 3-1000, The Johns Hopkins Hosp, Baltimore, MD 21205. Tel: 410-955-6463; Fax: 410-955-9773. S Raiti, MD, 5805 Stony Run Drive, Baltimore, MD 21210-1330.

June 3-6, 1993 3rd Intl Wkshp on Fetal Genetic Pathol, Perugia-Bosco, Italy. Info: Dr G Neri, Istituto di Genetica Medica, Universita Cattolica, Roma, Italy. Tel: +6-3054449; Fax: +6-3050031.

June 3-7, 1993 4th Joint LWPES/ESPE Mtg, San Francisco, CA. Info: For LWPES members: MM Grumbach, MD, Univ of CA-San Francisco, c/o Extended Programs in Medical Education, Room LS105, Box 0792, San Francisco CA 94143. Tel: 415-476-4251; Fax: 415-476-0318. For ESPE members: Dr M Ritzon, Dept of Paediatr Endocrinol, Karolinska Hospital, S-104 01, Stockholm, Sweden. Tel: 46-8-729-2465; Fax: 46-8-729- 5128.

June 9-12, 1993 75th Ann Mtg of The Endocrine Soc, Las Vegas, NV. Info: C Huck, The Endocrine Soc, 9650 Rockville Pike, Bethesda, MD 20814. Tel: 301-571-1835; Fax: 301-571-1869.

September 2-6, 1993 19th Ann Mtg of the Intl Study Group of Diabetes in Children and Adolescents (ISGD), Athens, Greece, on board MTS "Arcadia." Info: Dr C Bartsocas, Dept of Paediatr, "P & A Kyriakou" Children's Hosp, GR-11527 Athens, Greece. Tel: +30-1-7709316; Fax: +30-1-7796461.

September 6-8, 1993 Frontiers of Paediatr Neuroendocrinol, London, Eng. Info: Dr MO Savage, Dept of Endocrinol, St Bartholomew's Hosp, London EC1A 7BE, UK. Tel: 44-71-601-8487; Fax: 44-71-601-8505.

September 12-15, 1993 Ann Mtg of the Eur Soc for Paediatr Research (ESPR), Edinburgh, Scot. Sci Info: Prof NM McIntosh, Dept of Child Life and Health, Univ of Edinburgh, 17 Hatton Pl, Edinburgh EH9 1UW, Scot, UK. Tel: 44-31-667-2617; Fax: 44-31-668-2605. Genl Info: ESPR '93, Edinburgh Conf Ctr, Heriot-Watt Univ, Riccarton, Edinburgh EH14 4AS, Scot, UK. Tel: 44-31-449-5111; Fax: 44-31-451-3199.

September 13-15, 1993 Conference on "Glycobiology: New Perspectives on Human Disease." Info: G Holt, Natl Inst of Health, Bldg 10, Rm 9S242, 9000 Rockville Pike, Bethesda, MD 20892. Tel: 301-496-9101; Fax: 301-402-0234.

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Fragile X Syndrome: Review and Current Status

David L. Nelson, PhD

*Institute of Molecular Genetics
Baylor College of Medicine*

In the past year, a surprising new class of mutations involving unstable triplet nucleotide repeats has been found associated with 3 human genetic diseases. This article reviews recent findings in fragile X syndrome, the first of this type of unstable mutation to be described. Similarities and differences with myotonic dystrophy and spinal/bulbar muscular atrophy (Kennedy disease) are also noted.

Letter From the Editor

The **fragile X syndrome** has been an endocrine and genetic enigma. New and exciting gene findings prompt us to feature a lead article concerning the genetics of the syndrome and 3 abstracts concerning clinical findings. These all complement each other, which should enlighten you, our reader. You may wish to review Dr. Judith Hall's perspective, "The Strange Case of Fragile X Syndrome: Increased Mutation Frequency, Fragment Size, and/or Genomic Imprinting?" (*GGH* 1991;7[4]:9-10) before reading this issue. Dr. Hall's article sets the stage for the material contributed by Dr. David Nelson and the 3 abstracted journal articles. Please use your glossary included with the last issue of *GGH*. The asterisks (*) in Dr. Nelson's article indicate when you may be helped by referencing the glossary.

Robert M. Blizzard, MD

In This Issue

Abstracts	pg 5
Growth Hormone II: Basic and Clinical Aspects	pg 7
Meetings Calendar	pg 16

FRAGILE X SYNDROME

Fragile X syndrome is the most frequently encountered form of inherited mental retardation in humans, with a frequency estimated to be 1/1,250 males.^{1,2} Fragile X syndrome segregates as an X-linked dominant condition with incomplete penetrance* since either sex, when carrying the fragile X mutation, may exhibit mental deficiency. Sherman et al^{3,4} showed that approximately 30% of carrier females are affected and that 20% of males carrying the fragile X chromosome are phenotypically normal but may transmit the disorder and have affected grandsons. In addition to the mental retardation, which is variable in severity, affected males exhibit other manifestations, including macroorchidism and distinctive facies.⁵ Fragile X syndrome, as implied by the name, is associated with a fragile site,* expressed as an isochromatid gap in the metaphase X chromosome, at position Xq27.3.⁶

GENETICS OF FRAGILE X

Inheritance of the fragile X syndrome is quite complicated, although the features of the mutation at the DNA level (see below) are beginning to provide an explanation for the unusual genetics found in this disorder. The most striking aspect of fragile X is its incomplete penetrance in both males and females. This is particularly interesting in the case of normal transmitting males (NTMs), who transmit the mutation to grandsons but are unaffected themselves. More complicated are the probabilities of mental deficiency based on the affected status of relatives. This has become known as the Sherman paradox.^{3,4} It states that the probability of mental retardation is increased by the number of generations through which the mutation has passed. The probability is higher for both sons and daughters of affected females or females with affected sibs. The recent identification of the fragile X mutation, and its characterization as an unstable DNA fragment, provides a rational basis for this phenomenon.

THE FRAGILE SITE

In May of 1991, 3 groups reported identification of the fragile X site in Xq27.3 (Figure 1) using positional cloning* strategies.⁷⁻⁹ All made use of yeast artificial chromosome (YAC) clones containing this region to define a small section of the chromosome broken in a series of somatic cell hybrid lines.¹⁰ These hybrid cell lines contained chromosomal breakpoints suspected to be at the fragile X site. All groups found mutations in this chromosomal region in families with the fragile X syndrome. Somatic mosaicism in affected individuals was determined by inspection of DNA using Southern hybridization.* Two general classes of mutation were seen. These became known as *premutation* and *full mutation* and were first defined by increases in the size of fragments observed by Southern hybridization. Premutations are found in all NTMs and many carrier females, and involve increases in the length of this region by 50 to 500 bp. Full mutations are found in all affected individuals, male or female, and in some carrier females. The full mutation alleles show increases of 600 to 3,000 bp in length and are usually heterogeneous within an individual demonstrating somatic instability of the mutant allele.

The observation that a cluster of restriction sites in the fragile X region were differently methylated* in normal and fragile X males provided another clue to the location of the fragile site.^{11,12} Restriction enzymes that do not cut DNA when cytosines in their recognition sequences are methylated were used to demonstrate the differences.

This cluster comprised a tract of CG (CpG) dinucleotides known as a CpG island. CpG islands are often found near the transcription start sites* of genes. The specific methylation differences are observed in full mutations but not in premutation alleles.

THE FMR-1 GENE

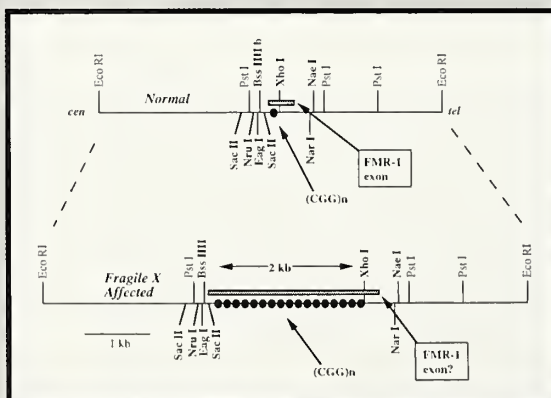
Verkerk et al⁷ also reported the isolation of a cDNA* from the region of the fragile X mutation. It was derived from a gene denoted FMR-1 (fragile X mental retardation-1). A 4.8-kb mRNA transcript from this gene was found in a variety of tissues, with brain tissue showing highest levels of expression. Additional unpublished results indicate that testes, uteri, and placentae all have similarly high levels of the transcript. Comparisons of the predicted amino acid sequence of FMR-1 have not revealed significant relationships with other known proteins. Related sequences have been observed by Southern hybridization in a variety of other species of mammals, as well as in yeast and *Caenorhabditis elegans*,* indicating that the FMR-1 gene has been highly conserved through evolution. The functions of FMR-1 are still unknown.

The most interesting feature of FMR-1 was the identification at the 5' end of a cDNA clone of the sequence (CGG)₅AGG(CGG)₉AGG(CGG)₁₀ (expressed hereafter as CGG repeats). In the reading frame* of FMR-1, this sequence would predict a run of 30 consecutive arginine residues. Such a sequence is unprecedented in known proteins, although there are a number of proteins with stretches of up to 10 arginine residues. These generally have DNA-binding activities (histones* and polyamines*). It is as yet unclear whether this sequence is translated into protein. However, this repeat sequence is the site of the fragile X mutations and accounts for their unstable nature.

Pieretti et al¹³ reported complete loss of expression of FMR-1 RNA in 80% (16/20) of fragile X males studied. The 4 cases with RNA expression demonstrated partial methylation of the CpG island, while the 16 cases with no expression showed complete methylation of the island. Thus, the expression of FMR-1 appears to be dependent upon the methylation status of the adjacent CpG island, and a likely mechanism of the fragile X phenotype involves expansion of the CGG repeats (see below) followed by methylation of the region, which causes loss of expression of FMR-1 RNA. The 4 cases with partial methylation were not detectably less severely affected. However, given the somatic mosaicism observed for the mutation, it is possible that the methylation pattern of blood cells studied was irrelevant since it did not reflect the pattern in cells actually responsible for the phenotype.

* Terms marked with an * are defined in the *GGH Genetics Glossary*, Volume 9, Number 1.

Figure 1
Map of the 5.2-kb Fragment in Xq27.3
Produced by Digestion With Restriction
Enzyme *EcoRI*



The fragment contains the CGG repeats (●) mutated in fragile X syndrome in normal and fragile X-affected forms. Restriction sites for other enzymes and the exon of FMR-1 are indicated. Restriction sites in bold type are sensitive to methylated cytosine residues in CpG dinucleotides. Cen refers to the centromere and tel to the telomere portion of the chromosome.

CGG REPEATS AND FRAGILE X MUTATION

While 2 of the 3 original reports noted this repeated sequence and suggested it may have a role in the unstable mutation, Kremer et al¹⁴ demonstrated that the site of mutation, as well as the instability, was within the CGG repeats, and that the size variation of restriction fragments observed by Southern hybridization was due to changes in the number of copies of the CGG repeats. Fu et al¹⁵ characterized the repeats in more detail, finding them to be polymorphic in the normal population, with the most frequent allele having 29 repeats and a range of sizes from 6 to 54 repeats. In fragile X premutations, the smallest number of repeats reported was 52, while the largest was 193; the majority were in the 75 to 120 range.

Precision of measurement of allele sizes in different persons was achieved by polymerase chain reaction (PCR)* amplification of the alleles followed by sequencing gel analysis for size discrimination. One provocative finding was the observation of instability of the premutation alleles during meiotic transmission. In every case examined (n=67), the number of repeats was found to be altered from parent to offspring. Thus, the mutation frequency of premutation alleles is ~10⁰. This is an unprecedented frequency. Mutations tend to increase the number of repeats in each generation; the number of repeats tends to grow upon passage from parent to offspring. Only 3 of 67 transmissions had decreases in size.

THE SHERMAN PARADOX

Fu et al also studied the frequency with which the premutation was altered to the full mutation from parent to child. With father-to-daughter transmission, the premutation expanded to the full mutation in none of the 4 examples. This also was seen in Southern analysis^{8,9,16} and fits with the observation that daughters of NTMs are never found to be affected.^{3,4} Premutation to full mutation changes are found only in offspring of females.

Recognition Award

With the support of Genentech, Inc. and the Editorial Board, *GROWTH, Genetics, & Hormones (GGH)* adopted the utilization of an environmentally conscious paper stock in 1991. We are pleased to share our Recognition Award from Mohawk Paper Mills, presented at the 1993 Editorial Board Meeting, for the utilization of 50/10 recycled stock. *GGH* was judged for this award against a multitude of publications with formats as varied and unique as the individual publications themselves. It is an honor to receive this award and we wish to thank our readership for your continued interest and to Genentech, Inc. for its generosity in supporting *GGH*.

The *GGH* Editorial Board

The risk of expansion to the full mutation varies with the size of the premutation in the mother. This finding, along with the observation of premutation and full mutation alleles, and the tendency of alleles to increase in size with subsequent generations, elucidates the Sherman paradox. The risk of retardation to an individual is dependent upon the number of repeats in his/her mother's allele. If a mother is affected, she already has an expanded full mutation allele (roughly one half of females with the full mutation are found to be affected).¹⁶ If this mother has an affected brother, she may have a premutation with a repeat number at the high end of the premutation range, or a full mutation that resulted in no phenotypic expression. In pedigrees with documented NTMs, however, the risks of retardation were found to be considerably smaller and the empiric data of Sherman fit nicely with the findings of Fu et al regarding escalating risks of expansion to the full mutation.

HERITABLE UNSTABLE DNA

Finding heritable unstable triplet repeats at the fragile X site led to speculation that similar repeats might be found in other conditions showing unusual inheritance patterns.¹⁷ One prime suspect was myotonic dystrophy, which has been mapped to a small region of chromosome 19. Myotonic dystrophy exhibits a phenomenon known as anticipation,* whereby the severity of the disease increases with subsequent generations. While there has been considerable debate about the existence of anticipation, the clue provided by the fragile X mutation prompted a search for triplet repeats in the relevant region of chromosome 19.

The Human Growth Foundation Announces a Grant Program

The Human Growth Foundation announces a Grant Program for investigation of human growth and its disorders. Special consideration will be given to new investigators and ideas new to the field. Research dealing with all aspects of normal and abnormal growth such as biologic, psychologic, educational, and dietary will be considered. One or more grants in the amount of \$7,500 to \$10,000 will be awarded.

An NIH-type biographic sketch and 2-page letter of intent should be sent to the address below by July 1, 1993. Applicants selected to submit a complete application will be notified by August 1, 1993, and completed applications will be due October 1, 1993.

Send correspondence to:

Human Growth Foundation
7777 Leesburg Pike, Ste. 202S
Falls Church, VA 22043

Numerous reports describing unstable DNA based on Southern hybridization,¹⁸⁻²⁰ and the subsequent identification of a triplet repeat (CTG in the 3' untranslated sequence of an mRNA likely encoding a protein kinase) as the basis of the instability have recently been published.²¹⁻²³ The severity of the phenotype in muscular dystrophy correlates well with the number of repeat units in the mRNA; and the number of repeats generally increases with subsequent generations, providing a molecular basis for the anticipation phenomenon. As in fragile X syndrome, the repeat is polymorphic. It ranges from 5 to 27 residues in normal individuals and grows in length to at least 50 repeats, from which it can expand dramatically in length. However, unlike fragile X, the repeat expansion in muscular dystrophy is found in offspring of both male and female parents.

A third human genetic disorder involving triplet repeats has been found. In spinal/bulbar muscular atrophy, the mutation has been localized to an increased number of CAG codons in a region of the androgen receptor gene on the X chromosome.²⁴ This region encodes a polyglutamine repeat in the receptor. It ranges from 17 to 26 residues in normal individuals, but exceeds 40 residues in affected individuals. Evidence for instability of the triplet repeat has not yet been published. As yet, no large expansions have been identified. The CAG repeat in spinal/bulbar muscular atrophy is the complement of the CTG repeat in muscular dystrophy; thus, these are the same repeats.

The identification of a mutation that confers increased mutability onto itself is a rather astounding finding, yielding insight into several previously mysterious phenomena in genetics. The idea that DNA is not necessarily inherited in the same form as the parents' or that it can be significantly altered from tissue to tissue within an individual is radical, and calls for reexamination of some of the principles of genetics. It is a rare delight when fundamentally new phenomena are uncovered in genetics, and heritable unstable DNA represents such a new principle. We can only hope that the continuing inquiry into the nature of our genes will yield more such insights.

CLINICAL TESTING FOR FRAGILE X SYNDROME

Advances in molecular testing for fragile X mutations have complicated the clinical evaluation of a child in whom the syndrome is suspected. Important questions surround the accuracy and reliability of both cytogenetic and molecular assays, especially with regard to the rare exceptions, such as deletions in the FMR-1 gene, that are not detected cytogenetically. Cytogenetic analysis certainly has a role in evaluating an isolated case of developmental delay, since other chromosomal abnormalities may be detected. However, in families with known fragile X mutations, it is difficult to justify the cost; DNA-based analyses may be

more accurate if they are available. There is still considerable debate about the merits of different types of DNA-based testing, ie, Southern hybridization-based versus PCR-based assays, and technical improvements continue to be made in many research and diagnostic laboratories. However, it is clear that DNA-based testing will be the method of choice for some time to come.

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In Future Issues

The Relevance of Developmental Genetics to Human Malformation

by Golder Wilson, MD

Overgrowth Syndromes and Disorders: Definition and Classification

by David Weaver, MD

The Overgrowth Syndromes: An Update

by Kenneth L. Jones, MD

Adrenarche and Its Variants

by Songya Pang, MD

The Importance and Methods of Using Animal Models to Study Human Disease

by Robin Winter, MD

Clinical Significance of Urinary GH Measurements

by Margaret MacGillivray, MD

Standard for Selected Anthropometric Measurements in Males With the Fragile X Syndrome

Butler et al prepared anthropometric data on 185 white males (ages 0 to 26 years) with fragile X syndrome confirmed by chromosome analysis. Height, weight, head circumference, ear length, and testicular volume were measured; similar control data were collected and utilized for comparison. Standards were then developed for the 5th, 50th, and 95th percentiles of both groups. At least 7 individuals were measured at each 1-year age interval.

The curves produced showed remarkable similarity in height and weight between fragile X subjects and controls, with the exception of a slight tendency for obesity in the affected individuals at approximately 12 years of age. Head circumference was slightly increased at all ages. Ear length at the 5th, 50th, and 95th percentiles was consistently above the respective values for normals at all ages (see Figure 1), as were testicular volumes. The 50th percentiles for testicular volumes approximated the control 95th percentile until 6 years of age, after which both the 50th and 95th

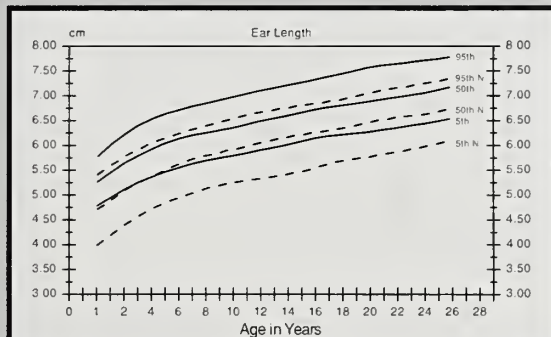
percentiles were markedly greater than the control 95th percentile (see Figure 2).

Butler MG, Brunschwig A, Miller LK, et al. *Pediatrics* 1992;89:1059-1062.

Editor's comment: The author states that fragile X syndrome is the most common genetic cause of mental retardation in males except for Down syndrome. The fragile X syndrome accounts for 30% to 50% of families with male mental retardation. The data presented in this article, in particular ear length and testicular volume, may be useful in identifying individuals for whom diagnostic chromosomal studies for fragile X are indicated. The etiology of these findings is not known.

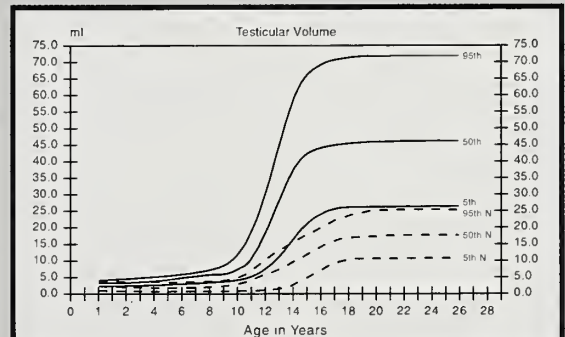
William L. Clarke, MD

Figure 1



Standardized curves for ear length of males with fragile X syndrome (solid line) and normal individuals (broken line).

Figure 2



Standardized curves for testicular volume of males with fragile X syndrome (solid line) and normal individuals (broken line).

Psychiatric Disorders Associated With Fragile X in the Young Female

Females heterozygous for fragile X chromosomal variations are usually less affected than males, with only 35% exhibiting mental retardation. Studies by others have demonstrated specific short-term memory deficits, characteristic Wechsler IQ test subtest profiles, and frontal lobe deficits, suggesting verbal reasoning strengths and visual-spatial deficits in fragile X girls. Freund et al evaluated the prevalence of psychiatric and behavioral disturbances among a group of 17 fragile X females. This group was matched to 17 non-fragile X females who were similar in age, IQ, and socioeconomic status. All fragile X girls had cytogenetically confirmed fragile X syndrome. Cytogenetic testing was available on 12 of the 17 controls. The age range of the fragile X girls was from 4 to 27 years; that of the control group from 7 to 27 years. IQs averaged 78.2 in the fragile X group and 80.5 in the control group. Socioeconomic status ranged from lower to upper middle categories in both groups.

The Diagnostic Interview for Children and Adolescents-Parent version was administered to parents to ascertain psychiatric diagnoses. These diagnoses were based on *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition, Revised (DSM-III-R)

criteria, and included additional modifications for depression in the developmentally disabled. Adaptive behavior was assessed utilizing the Vineland Adaptive Behavior Scales, Survey Form. This assessed behaviors in 3 domains including communications, daily living skills, and socialization. The Revised Behavior Problem Checklist was given to parents and teachers to assess problem behaviors, including conduct, socialized aggression, attention problems, anxiety-withdrawal, psychotic behavior, and motor-tension excess. Cognitive assessment was performed with either the Stanford-Binet Intelligence Scale, Fourth Edition, or the Wechsler Intelligence Scale for Children-Revised.

Fragile X females have significantly more avoidant disorder of childhood and adolescence (ADCA) or avoidant personality disorder (65%), more mood disorders (47%), and more stereotypy-habit disorders (35%) than the control group. The majority of the fragile X girls (5 of 8) with a current or past mood disorder also met the criteria for ADCA. Stereotypy included repetitive smelling of objects, hand biting, excessive nail biting, hand clapping, or head banging. The frequency of stereotypy with ADCA and/or mood disorder was 83%. Female fragile X subjects also demonstrated

significantly lower socialization scores, especially in the area of interpersonal skills, eg, a lack of friends, as well as difficulty initiating, maintaining, and ending social conversations appropriately. Both parents and teachers report higher scores on the anxiety-withdrawal scale, behaviors which would include being shy, easily embarrassed, fearful, depressed, and sad.

DNA insert size was determined for 13 of the fragile X females and a significant correlation was shown between the size of the insert and IQ. In addition, the anxiety-withdrawal scores correlated positively with base-pair insert size when controlling for IQ, ie, increasing insert size correlated with increasing severity of anxiety and withdrawal behaviors.

Freund LS, Reiss AL, Abrams MT. *Pediatrics* 1993;91(2):321-329.

Editor's comment: Findings from this paper suggest that there

may be a significant prevalence of psychiatric disability in fragile X females and that the increase in size of the fragile X DNA insert may be associated with lower IQ and increased severity of anxiety-withdrawal symptoms. These associations are intriguing even though the pathogenetic mechanisms remain obscure. The authors have pointed out the significant limitations of their study, which include a small sample size, a wide age range, the lack of DNA testing on control subjects, and broken blindness to group membership. Despite these limitations, this study provides important information to the clinician dealing with these patients and their parents as it suggests that significant psychiatric disorders may occur. Whether or not such disorders will be amenable to treatment is not known. The recognition that certain behaviors may occur in fragile X females points out the need for prospective evaluations.

William L. Clarke, MD

Girls With Fragile X Syndrome: Physical and Neurocognitive Status and Outcome

Male patients with fragile X syndrome have been the primary research focus as the entity is X-linked; and males, therefore, are more severely affected than females. Hagerman et al broadened the study of the fragile X syndrome to females. Thirty-two fragile X positive girls, 1 to 18 years of age, were compared with their sisters (n=19) who were fragile X negative. Some of the latter may have carried the gene.

Phenotypic features of affected individuals are listed in Table 1. A characteristic facial appearance is presented in Figure 1. A physical index score was obtained by adding up the number of phenotypic abnormalities listed in Table 1. Three of 32 girls had no phenotypic characteristics, 2 girls had 6 of the phenotypic abnormalities, and the remainder had between 1 and 5 phenotypic abnormalities.

Intellectual learning and behavior difficulties were frequent and included hyperactivity, shyness, hand flapping, poor eye contact, tactile defensiveness, impulsivity, and distractibility. Intelligence evaluations revealed an IQ <70 in 25% and <84 in 53% of the girls. No correlation between the extent of X fragility and IQ levels was found. However, the percent fragility statistically correlated (although not strongly) with the physical index score. IQ did not decline with age, which has been reported in boys with fragile X syndrome.

The authors recommended cytogenetic testing of all female siblings of fragile X males.

Hagerman RJ, Jackson C, Amiri K, et al. *Pediatrics* 1992;89:395-400.

Editor's comment: Fragile X syndrome does occur in girls. While patients with this syndrome frequently have both developmental and phenotypic characteristics, either may occur alone. Evolution of our knowledge about this syndrome is fascinating. It remains to be seen if imprinting is involved.

Robert M. Blizzard, MD

Table 1
Physical Features Associated With Fragile X Syndrome

Phenotypic Feature	(n = 32) X Positive %	(n = 19 sibs) X Negative %
Long ears (measurement from top to bottom of pinna)	12.9	5.6
Prominent ears (subjectively estimated as visually prominent)	56.3	11.8
Long narrow face (subjectively determined)	46.4	7.7
High arched palate (subjectively determined)	19.4	5.9
Hyperextensible meta- carpal phalangeal joints	62.1	38.9
Double-jointed thumbs	33.3	22.2
Hand calluses (from hand biting)	3.3	0
Simian crease	21.4	6.3
Flat feet	36.7	23.5
Murmur or systolic click	13.3	0

Figure 1



Two sisters who are fragile X positive. Note prominent ears in both and long narrow face of sister on the left.

Growth Hormone II: Basic and Clinical Aspects

*Conference Summary: Tarpon Springs, Florida,
December 3-6, 1992*

Chairmen: B.B. Bercu, MD, and R.F. Walker, MD

Allen W. Root, MD

Recent developments in the regulation, physiology, and metabolic effects of growth hormone (GH) were discussed at this conference. Reisine et al and Coy et al described the family of 5 somatostatin (SRIH) membrane receptors identified by their differential binding of various analogues of SRIH and distinguishing modes of regulation and mechanism(s) of action. SRIH receptor-4 is expressed only in the anterior pituitary. Kraicer and Sims reviewed the evidence indicating that GH-releasing hormone (GHRH) acts through a G-protein to increase adenylyl cyclase activity and intracellular levels of cyclic AMP, leading to activation of protein kinase A, increased intracellular Ca^{++} and exocytic release of GH. SRIH inhibits influx of Ca^{++} and release of GH. Melmed described the paracrine inhibitory effect of pituitary insulin-like growth factor 1 (IGF-1) on GH gene transcription and GH secretion. IGF-1 (synthesized in the pituitary folliculostellate cell in response to GH, cortisol, and triiodothyronine and acting through type 1 IGF receptors in the somatotroph membrane) can inhibit the stimulatory effects of GHRH, cyclic AMP, and triiodothyronine on GH gene expression.

Blalock reported that the synthesis and secretion of lymphocyte-derived GH are regulated by GHRH and IGF-1, but not by SRIH or GH itself. Lymphocyte GH stimulates lymphocyte production of IGF-1 and lymphocyte proliferation. Kelley detailed the many actions of GH and IGF-I on the immune system including an increase in macrophage and neutrophil production of superoxide anion — necessary for antimicrobial activity. In human neutrophils, the action of GH is mediated through the prolactin receptor (binding to this receptor is aided by Zn^{++}). Mulligan et al administered GH to 6 men with the acquired immunodeficiency syndrome (HIV+) for 7 days and observed nitrogen retention, decreased protein and increased lipid oxidation, and other anabolic effects. Further study will be required before the role of GH in this and other wasting diseases is determined.

The effects of GH in the aged human and experimental animal were a focus of interest. Rudman reported on extended studies in elderly hyposomatomedinemic (IGF-I <0.35 U/mL) men; after 12 months of GH administration lean body mass had increased to 106% and body fat had decreased to 84% of baseline values. However, among 61 GH-treated subjects, 10 developed carpal tunnel syndrome, 4 gynecomastia, and 3 hyperglycemia. The likelihood of an adverse event occurring

during GH administration was greater if IGF-1 concentrations exceeded 1 U/mL during therapy. Although serum concentrations of IGF-binding protein (IGFBP-3), osteocalcin, and the propeptide of type 1 collagen increased, Marcus et al noted no effect of GH administered for 12 months to healthy elderly women (not specifically selected for low IGF-1 levels) on bone mineral density, lean body mass, or fat composition (by hydrostatic weighing, although fat estimated by skin-fold thickness declined, an effect attributed to fluid retention inasmuch as it occurred within 7 days of initiation of treatment). Thus, any beneficial effects of GH administration to the elderly have yet to be demonstrated conclusively.

The development of several small peptides (6 to 7 amino acids) that stimulate the secretion of GH was discussed by Bowers et al. These peptides are effective when administered parenterally or orally. They stimulate release of GH by a cyclic AMP-independent mechanism, and thus act in a manner different than that of GHRH. Consequently, the GH-releasing peptides (GHRP) act synergistically with GHRH. Bowers hypothesized that the endogenous GHRP ligand (that has yet to be identified but may be related to the endogenous opiate family of peptides) may primarily act to amplify the effect of endogenous GHRH on GH secretion. Chihara et al reported that in short children, a 6-amino-acid GHRP-6, administered as an intravenous bolus injection stimulated GH release in a manner quantitatively similar to that of GHRH and greater than that following insulin hypoglycemia or levodopa, but was more reproducible than was GHRH. Walker reported that the combined administration of GHRP-6 and GHRH to aging female rats for 60 days lead to significantly lower pituitary, adrenal, and kidney weights, and a decreased incidence of pituitary adenomas compared to saline-treated aged control animals. Plasma concentrations of cholesterol were reduced in the GHRP-6/GHRH-treated animals. (A non-peptidyl, small molecule that also stimulates GH release when given orally was described by Schoen et al - Merck.)

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Growth Hormone Secretion in Turner's Syndrome

Twenty-four growth hormone (GH) profiles in 26 girls with Turner syndrome were compared with those of 26 normally growing short children (18 males, 8 females) and 24 slowly growing short children (17 males, 7 females). All patients studied were prepubertal and less than 12 years of age (study 1). A randomly selected subgroup of 13 Turner girls was restudied during treatment with ethinyl estradiol 0.05 µg/kg/d. Separate samples were obtained, and GH was measured by immunoradiometric assay (IRMA).

A second trial (study 2) was done with 45 girls with Turner syndrome, aged 6.7 to 18.9 years, submitted to continuous blood sampling. A different IRMA kit was used for GH measurements. These patients were divided into 4 subgroups:

1. age less than 12 years, no treatment;
2. age more than 12 years, no treatment and no spontaneous breast development;
3. age more than 12 years, spontaneous breast development; and
4. age more than 12 years, treated with ethinyl estradiol 0.1 µg/kg/d.

Time series analysis of the results was done by Fourier transformation. In addition, the mean GH level of each profile was used for estimation of the differences between groups and for correlation with clinical situations.

In study 1, the mean 24-hour serum concentrations of the Turner girls and of the normally growing short children were both significantly higher than those of the slowly growing short children ($P < 0.0001$). In the Fourier analysis, the dominant periodicity of GH secretion was similar in the 3 groups of children, but the oscillatory activity was lower in the slowly growing children, resulting in a reduced spectral power. Estrogen treatment significantly increased the pulse amplitude but did not change the periodicity.

In study 2, the estrogen-treated Turner girls had a higher mean GH than the others, but the difference was not significant. Fourier analysis did not show significant differences between the 3 subgroups of patients over 12 years of age. There was no relationship between mean 24-hour GH levels and age. Linear regression analysis did not show a relationship between the height (standard deviation scores for Turner references and for bone age) and the mean 24-hour level of GH.

Thus, the authors point out that the regulation of GH pulse amplitude and frequency is normal in girls with Turner syndrome. This clearly shows that short stature in Turner syndrome is not related to insufficient or abnormal GH secretion. The findings agree with clinical therapeutic studies, which suggest that girls with Turner syndrome are relatively resistant to GH treatment and need high doses of GH for improving their growth rate.

Wit JM, Massarano AA, Kamp GA, et al. *Acta Endocrinol* 1992;127:7-12.

Editor's comment: The first trials of GH treatment in Turner syndrome were largely related to the reported finding by some groups of reduced release of GH, mainly after the age of 10 to 12 years. Further clinical experience clearly showed that the results of treatment with GH in these patients were in no way related to the results of any measurement of GH secretion. This sophisticated study gives clear confirmation that short stature in Turner syndrome, at least up to adolescence, does not result from abnormal or insufficient secretion of GH. This study may be of importance for future understanding of the short- and long-term effects of GH treatment in Turner syndrome, a very peculiar model of short stature with low biologic GH sensitivity but acceptable responses to supraphysiologic GH doses.

Jean-Claude Job, MD

Predictive Factors for the Effect of Gonadotrophin Releasing Hormone Analogue Therapy on the Height of Girls With Idiopathic Central Precocious Puberty

Brauner et al studied 14 girls with idiopathic central precocious puberty (CPP) treated with the gonadotropin-releasing hormone (GnRH) analogue Decapeptyl (a long acting preparation of D-Trp⁶ GnRH analogue) beginning at a mean age of 7.1 ± 0.4 years. The mean age at the onset of puberty was 5.7 ± 0.4 years. Growth hormone secretion was evaluated by arginine-insulin infusions and was within normal limits in all patients. Bone age was evaluated by the atlas of Greulich and Pyle; target height was calculated by the method of Tanner; and final height was predicted according to Bayley-Pinneau tables. The mean target height was 161.8 ± 1.4 cm, but the mean predicted height prior to the onset of therapy was 152 ± 1.8 cm. GnRH therapy (3.5 mg IM every 25 days) was given for a mean duration of 3.1 ± 0.3 years and stopped either at the request of the patient or when the bone age was more than 12 years. The mean follow-up after cessation of therapy was 1.4 ± 0.2 years.

Estrogen activity was fully suppressed during therapy, as determined by both basal and GnRH-stimulated plasma luteinizing hormone and follicle-stimulating hormone. The mean bone age increased from 10.6 ± 0.2 years to 12 ± 0.1 years over a mean of 3.1 ± 0.3 years. The mean predicted final height increased from 152 ± 1.8 cm at the onset of therapy to 160.4 ± 1.3 cm at the end of therapy and to 162.2 ± 1.2 cm at a mean of $1.4 \text{ year} \pm 0.2 \text{ years}$

after the cessation of therapy. Thus, the mean total gain in predicted height was 10.2 ± 1.1 cm. The authors demonstrated that individual gains correlated positively with bone age advance over chronologic age ($r = 0.66$, $P < 0.02$) and with the difference between target height and pretherapy predicted height ($r = 0.76$, $P < 0.001$) and negatively with the height predicted before therapy ($r = -0.76$, $P < 0.001$). Height gains were not correlated with either chronologic or bone age at the onset of therapy or with the duration of therapy.

Brauner R, Malandry F, Rappaport R. *Eur J Pediatr* 1992;151:728-730.

Editor's comment: At first glance, these data may be reassuring to the pediatric endocrinologist treating girls with idiopathic CPP with long-acting GnRH analogues. The data suggest that predicted height can be increased as much as 20.5 cm (1 patient); however, final height has not yet been achieved in this group. Thus, although it would appear that growth has continued at normal rates following cessation of therapy, the final data will not be available until growth has ceased in all of these girls. The authors state that the therapeutic effect of GnRH therapy on improving predicted height is best in those with the most accelerated bone ages at the onset of therapy, the lowest initial predicted heights, and the largest

difference between the target height and the initial predicted height. These would appear to be the girls with the most severe disease (final height most affected by their sexual precocity). However, there are some caveats that should be acknowledged. Bone age was never greater than 12 years in any of these patients until discontinuation of GnRH therapy, and bone age of 12 years was the criterion for discontinuation of therapy. Even though the mean advance of bone age and the mean bone age increase are reported, it is important to view the raw data, including the actual range of initial bone ages, to interpret the findings. Clearly a short girl with a bone age of 11 years 6 months would have had little

height benefit during this study, since therapy would have stopped at a bone age of 12 years. In addition, it would be important for the authors to calculate, using stepwise regression analysis, the contribution of each of these variables to the variance in the improvement in predicted height. Thus, the paper provides tantalizing information, but the data are insufficient to answer the question of what factors predict the effect of GnRH analogue therapy on the height of girls with idiopathic CPP. I invite comments through the Letter to the Editors column regarding my deductions.

William L. Clarke, MD

Autoimmune Addison's Disease: Enzymes as Autoantigens

Two adrenocortical steroid biosynthetic enzymes (17 α -hydroxylase and 21-hydroxylase) have been recognized as autoantigens in patients with autoimmune adrenocortical insufficiency (Addison's disease). Krohn et al¹ identified antibodies against 17 α -hydroxylase (P450c17 α) in the sera of children with the autosomal recessive type-1 polyendocrine autoimmune syndrome (PAS.I), which consists of at least 2 of 3 entities (hypoparathyroidism, chronic mucocutaneous candidiasis, and Addison's disease). Hypogonadism, vitiligo, alopecia, and pernicious anemia also are often present. Precipitating antibodies against human adrenal homogenates were found by immunodiffusion in 21 of 35 subjects with PAS.I. Further analysis of sera by western blotting revealed antibodies against several adrenal proteins with molecular weights ranging from 19 to 55 kd. Antibodies against the 55 kd protein were present only in the sera of patients with precipitating antibodies detected by immunodiffusion. The 55 kd autoantigen proved to be the P450c17 α protein, as the cDNA of this protein was 98.8% homologous with the gene for P450c17 α . Vector expression of this cDNA resulted in a protein recognized only by serum immunoglobulins from PAS.I subjects. The sera of 2 adult patients with only idiopathic Addison's disease did not contain antibodies against P450c17 α .

Winqvist et al² found antibodies against 21-hydroxylase (P450c21) in the sera of 12 of 16 adults with isolated Addison's disease, all of whose sera contained immunoglobulins reacting most strongly with the outer zona glomerulosa of the adrenal cortex by immunofluorescence. Immunoblotting techniques revealed that the autoantigen identified in the sera of these 12 patients comigrated with the P450c21 protein, but not with the proteins of the side-chain cleavage enzyme (P450scc), 11 β -hydroxylase (P450c11 β), or P450c17 α . Preabsorption of P450c21 protein with a rabbit antiserum to this protein abolished the reactivity of the patients' sera with the adrenal antigen, whereas preabsorption with antisera to P450scc, P450c11 β , or P450c17 α proteins did not. Baumann-Antczak et al³ confirmed these findings.

steroidogenic enzymes may be autoantigenic. In adult subjects with isolated adrenocortical insufficiency, antibodies against P450c21 were predominant. However, in 4 of 16 patients with antiadrenocortical antibodies studied by Winqvist et al the adrenal autoantigen was not identified, again indicating the immunoheterogeneity of this disorder. The enzyme P450c17 α is expressed in the adrenal cortex, ovary, and testis; P450c21 is expressed only in the adrenal cortex. This suggests that antibodies to these enzymes may be of pathogenetic significance in the different patterns of the 2 diseases, therefore, possibly associated with gonadal and adrenal failure.

The reason that different steroidogenic enzymes prove antigenic in differing forms of autoimmune Addison's disease is unknown; this observation requires confirmation and investigation of the genes and the processing of their products in the 2 disorders. If the enzyme antibodies are of pathophysiologic importance in these disorders, rather than secondary manifestations of the primary abnormality, their mechanism(s) of action are unclear. The antibodies might adversely affect enzyme function, but the manner in which the antibodies enter the cell and gain access to the cytoplasmic reticulum or microsomes is not known. If these antibodies fix complement, they also might act through a complement-mediated insult.

Thus, antibodies against 3 adrenocortical steroidogenic enzymes present in the sera of subjects with autoimmune Addison's disease join the list of autoantibodies to enzymes identified in other autoimmune diseases (autoimmune thyroid disease: thyroid peroxidase; diabetes mellitus type 1: glutamic acid decarboxylase; autoimmune gastritis: H⁺, K⁺ adenosine triphosphatase; autoimmune hepatitis type II: cytochrome P450db-1).

Allen W. Root, MD

1. Krohn K, Uibo R, Aavik E, et al. *Lancet* 1992;339:770-773.
2. Winqvist O, Karlsson FA, Kämpe O. *Lancet* 1992;339:1559-1562.
3. Baumann-Antczak A, Wedlock N, Bednarek J, et al. *Lancet* 1992;340:429-430. Letter.
4. Enzymes as autoantigens. *Lancet* 1992;339:779-780. Editorial.

Editor's comment: These reports demonstrate that immunoglobulins against steroidogenic enzymes may develop in subjects with autoimmune Addison's disease. In patients with PAS.I, the most common antibody was directed against P450c17 α , but Winqvist et al also identified 1 subject with PAS.I who had antibodies against P450scc, suggesting that in this syndrome,

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The Androgen Receptor Gene in Androgen Insensitivity Syndromes

This study has used restriction fragment length polymorphism (RFLP) analysis of DNA for studying a large group of 52 patients with karyotype 46,XY and androgen insensitivity syndrome, considered as complete in 27 males having a female phenotype, and as partial in 25 males having ambiguous external genitalia. Endocrine investigation of these patients showed concomitantly high plasma testosterone and luteinizing hormone levels. The 52 patients were followed in 20 different clinics. Twenty-one were familial and 31 were isolated cases. Androgen-binding studies were performed from cultures of genital skin fibroblasts. Genomic DNA was prepared from peripheral blood leukocytes. DNA samples were studied by Southern blot analysis after digestion by 4 different restriction enzymes and hybridization with 3cDNA probes covering the 3 domains of the androgen receptor. The informative relatives of the probands, ie, their mothers and unaffected brothers, were studied to the extent it was possible.

Androgen-binding studies revealed that androgen receptor-binding capacity was undetectable in the 27 patients with complete androgen insensitivity. In the 25 patients considered on a clinical and hormonal basis as having partial insensitivity, receptor-binding capacity ranged from 120 to 340 fmol/mg DNA (normal mean, 650 ± 200 fmol/mg DNA), with dissociation constants in the normal range of 0.5 ± 0.25 nM. Thus, androgen-binding studies did not sustain the clinical and hormonal evidence of partial androgen insensitivity.

No large DNA deletion was found in any of the 52 patients. This suggests that in these studies of androgen insensitivity syndromes, abnormalities of androgen receptor could be related to point mutations or to microdeletions, rather than to gross alterations of the receptor gene.

Heterozygosity in the mother was found in 3 of 14 families studied with the *HindIII* polymorphism, and in 12 of 25 families using the exon 1 CAG repeat polymorphism. This suggests that *HindIII* and exon 1 polymorphism studies would be of considerable help in prenatal diagnosis of androgen insensitivity in male fetuses and in identification of carrier females, at least for half of affected families.

Lobaccaro JM, Belon C, Chaussain JL, et al. Molecular analysis of the androgen receptor gene in 52 patients with complete or partial androgen insensitivity syndrome: a collaborative study. *Horm Res* 1992;37:54-59.

Editor's comment: This genetic approach to the androgen insensitivity syndromes is a considerable work since it is based on multicenter clinical trials. It provides a complete and accurate biochemical study of androgen receptors in genital skin cells and studies of genomic DNA in white blood cells. The first and most important fact is that whatever the degree of clinical androgen insensitivity and lack of cellular androgen receptivity, no large genomic deletion has been found in the many patients studied. Thus, the microdeletions and/or point mutations responsible for androgen insensitivity are still to be elucidated. The second fact is that, even in these conditions, RFLP techniques allow for familial studies in both complete and partial androgen insensitivity syndromes, offering a reasonable chance to detect the carrier females after the study of one index case. Therefore, the possibility of prenatal diagnosis is offered. It is an important step in the complicated and difficult field of the genetics of androgen insensitivity syndromes.

Jean-Claude Job, MD

Growth Hormone Deficiency in Down's Syndrome Children

In this study, the capacity to secrete growth hormone (GH) was investigated in 20 children with Down syndrome (DS) to determine if GH deficiency plays a role in growth retardation in DS. The subjects (13 boys, 7 girls) were aged between 15 months and 13.9 years, had a height standard deviation score (SDS) ranging from -1.19 to -5.48, a weight SDS of -0.21 to -4.58, and a head circumference SDS from -0.4 to -6.6 below the mean for normal children of the same age and sex. All but 1 severely mentally retarded child attended infant stimulation programs. All but 2 subjects had moderate to severe expressive language impairment and were institutionalized from early infancy. GH was evaluated in all 20 patients by levodopa (125 mg up to 15 kg, and 240 mg between 15 to 30 kg) and clonidine (0.15 mg m^{-2}) stimulation tests. GH secretory patterns were assessed in 4 patients via integrated 24-hour GH concentration (IC-GH) using a constant withdrawal pump with continuous blood collection every 30 minutes. Normal IC-GH values were considered to be above 3.2 ng/mL. Peak serum GH after levodopa and clonidine stimulation was found to be below 10 ng/mL for both tests in 7 of the 20 children studied. Twelve children showed a disparity between levodopa and clonidine testing. Peak serum GH after levodopa administration was found to be below 10 ng/mL in 5 children; and peak serum GH following administration of clonidine was found to be below 10 ng/mL in 7 children. Four children had reportedly abnormal 12- or 24-hour IC-GH, with mean values below 1.5 ng/mL. These 4 subjects had previously shown GH levels above 10 ng/mL in at least 1 of the

stimulation tests. Additional endocrine testing revealed no thyroid or prolactin abnormalities in any of these patients; serum luteinizing hormone, follicle-stimulating hormone, and testosterone levels were appropriate for age; and insulin-like growth factor 1 (IGF-1) levels were normal in all DS patients.

The authors conclude that the growth retardation observed in these DS children was associated with a reduced serum GH response to levodopa and clonidine stimulation tests, disparity in responses to the stimulatory tests, and low 24-hour IC-GH.

Castells S, Torrado C, Bastian W, et al. *J Intell Disabil Res* 1992;36:29-43.

Editor's comment: This study invites us to think that reduced GH secretion plays a role in growth retardation in DS. The authors speculate that DS children have fewer neurons and neuronal connections in the CNS, which accounts for the abnormalities in GH secretion found. However, as the authors pointed out, the discriminator of 10 ng/mL for peak serum GH responses to provocative stimuli is rather arbitrary. Decreased levels after stimulation tests (false-negatives) and disparity among test responses are well known to occur even in normal short children. In these studies, the peak response was between 7 and 10 ng/mL for levodopa in 2 patients, for clonidine in 3 patients, and for both levodopa and clonidine in another patient. The authors did not have their own control values for GH levels. In many laboratories using the same GH kit (Quintapace; Kallestad Inc., Austin, Texas) used in this study,

values between 7 to 10 ng/mL could be considered adequate and would rule out GH deficiency. Moreover, some IC-GH measurements were made with the Hybritech Tandem kit, which records lower GH levels than many other assays. Finally, all of these DS patients had normal IGF-1 levels for age. Therefore, the patients did not meet biochemical criteria for classic GH deficiency to account for growth failure. Unfortunately, only 4 of the patients who had appropriate responses to at least 1 pharmacologic stimuli had IC-GH measured, all 4 being abnormal. There were no such measurements made in the other 16 patients described in this report. Thus, no conclusion should be derived from these very few cases that the etiology of growth failure in DS patients results from inadequate spontaneous GH secretion.

A major pitfall of the study is the fact that obesity was not considered in the equation. Most of the DS patients were obese, as evidenced by the weight and height data; the majority of them were overweight for height. Since obesity is known to cause hyporesponsiveness of GH secretion to provocative stimuli and to reduce IC-GH, it is very possible that these obese DS patients could have responded normally after priming with pyridostigmine,

as has been demonstrated in obese normal children.¹ Elsewhere these authors reported data that showed benefits of GH therapy in DS patients.² (This information was reviewed previously in GGH.)³ Caution must be exercised in obtaining incomplete data and extrapolating results of GH testing to establish the diagnosis of GH alterations in DS to justify treatment with GH. Other genetic mechanisms that relate directly or indirectly to gene abnormalities of chromosome 21 may be most important in determining height in DS patients.

Fima Lifshitz, MD

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2. Torrado C, Vastian W, Wisniewski, et al. Treatment of children with Down syndrome and growth retardation with recombinant human growth hormone. *J Pediatr* 1991; 119:478-483.
3. Lifshitz F. *Growth, Genetics, and Hormones* 1991;8(1):15.

Constitutional Delay of Growth and Adolescence: Results of Short-Term and Long-Term Treatment With GH

Bierich et al report final adult heights of children with constitutional delay of growth and adolescence (CDGA) who received exogenous growth hormone (GH) therapy and compared this data to the predicted heights prior to treatment. Thirteen boys and 2 girls were studied. Prior to treatment, nocturnal GH secretion was measured and shown to be approximately half that of a control group. In addition, the mean peak growth hormone levels following IV arginine also were half that of normal controls. All of the children were below the 3rd percentile for height, and their bone ages, according to Greulich and Pyle, were retarded by more than 2 years. Height predictions, by the method of Bayley and Pinneau, were performed at the initiation and termination of GH treatment and target height was determined according to the method of Tanner. Duration of GH therapy ranged from 2.5 to 6 years (mean, 3 years); 7 of the children were in early puberty at the start of treatment.

The mean final height of these 13 children was not different from the mean predicted height prior to GH treatment, but was significantly less than the target height. Although the authors did not include a control group for comparison, a meta-analysis of 5 studies of untreated CDGA children was performed. The mean final height of these children was 168.7 cm, while their predicted height was 170.4 cm and their target height was 172.8 cm. No statistics were provided, but it would appear that these differences were not significant. The authors concluded that children with CDGA become relatively short adults, when compared with their parents, regardless of whether they receive GH therapy. They further suggested that studies are needed in which GH therapy is continued until the epiphyses are closed. Bierich et al remind us that similar increases in height may be produced with oral oxandrolone or testosterone, if sexual development is a particular problem.

not affected. These findings are not unexpected, but are important in that some physicians may interpret the prepubertal growth deceleration in children with CDGA as an indication for GH therapy. This paper would suggest that this is not so. It is unclear why GH therapy was discontinued for the patients in this study, but restoration of height deficit (increased height standard deviation scores), may have been the indicator for discontinuance. It would have been interesting to have continued treatment in these individuals until epiphyseal fusion. It is unclear whether such studies are presently underway. The cost of GH therapy is not comparable to that of oxandrolone or testosterone. If similar results can be attained with these drugs, there is little justification for GH treatment of children with CDGA.

William L. Clarke, MD

2nd Editor's comment: Although GH treatment in delayed adolescence was once considered as useful based on good short-term results, this study could be interpreted to suggest that GH has minimal effect on final height. This paper, by a group that previously advocated GH in such situations, is to be considered both an interesting contribution to the management of growth delay as well as an example of fair self-evaluation and honesty in this field.

Jean-Claude Job, MD

Bierich JR, Nolte K, Drews K, et al. *Acta Endocrinol* 1992;127:392-396.

Editor's comment: This paper confirms previous findings that children given GH will respond with an increase in growth velocity, but suggests that, at least in the group with CDGA, final height is

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Insulin-Like Growth Factor 1 Improves Glucose and Lipid Metabolism in Type 2 Diabetes Mellitus

Type 2 (non-insulin dependent) diabetes mellitus (DM) is associated with hyperinsulinemia and a degree of insulin resistance. In order to determine the effect of insulin-like growth factor 1 (IGF-1) in patients with type 2 DM, the investigators administered recombinant human IGF-1 (120 µg/kg body weight per dose) by twice daily SC injection for 5 days to 8 adults (2 females) with type 2 DM. During IGF-1 administration: total and free IGF-1 concentrations increased as anticipated; IGF-2 and basal growth hormone (GH) concentrations fell; fasting glucose, fructosamine, triglyceride, insulin, C-peptide, and proinsulin levels declined; postprandial concentrations of glucose, insulin, C-peptide, and proinsulin, and the insulin:glucose and proinsulin:insulin ratios fell. Basal concentrations of glucagon were not changed by IGF-1 administration. The decline in fasting concentrations of glucose, triglyceride, insulin, and C-peptide during treatment with IGF-1 correlated directly with their respective fasting control levels.

The authors suggested that IGF-1 in type 2 DM:

1. decreased glucose concentrations by interaction of free IGF-1 and/or IGF-1 bound to IGF-binding protein 1 with type 1 IGF and insulin receptors in muscle. (Free IGF-1 levels are increased by lower insulin values and, when combined with IGFBP-1, cross the vascular barrier more easily than does IGF-1 bound to IGFBP-3.);
2. suppressed insulin secretion by a direct effect on the pancreatic beta cell; and
3. improved insulin sensitivity by lowering glucose, insulin, GH, and triglyceride concentrations.

They concluded that IGF-1 may have a therapeutic role in the management of patients with type 2 DM.

Zenobi PD, Jaeggi-Groisman SE, Riesen WF, et al. *J Clin Invest* 1992;90: 2234-2241.

Editor's comment: This article complements others that report the beneficial effects of IGF-1 in insulin resistant states, such as type 1 (insulin-dependent) DM.¹ In patients with type 2 DM, IGF-1 probably lowered glucose values by increasing glucose transport into muscle, acting through IGF-1 and/or insulin receptors stimulated by free IGF-1 and IGF-1 bound to IGFBP-1 that crossed vascular barriers and then dissociated from rapidly degraded IGFBP-1. It is likely that IGF-1 may have important therapeutic potential in insulin-resistant states as may an incompletely processed form of pro-IGF-2.²

Allen W. Root

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2. Zapf J, Futo E, Peter M, Froesch ER. Can "big" insulin-like growth factor-II in serum of tumor patients account for the development of extrapancreatic tumor hypoglycemia? *J Clin Invest* 1992;90:2574-2584.

Growth Hormone Deficiency During Puberty Reduces Adult Bone Mineral Density

Hyer et al measured bone mineral density (BMD) by dual energy X-ray absorptiometry in 60 adults (aged 23 to 76 years) with growth hormone deficiency (GHD, defined as a peak GH response below 5 mU/L after insulin-induced hypoglycemia). Ten of the 60 patients had GHD documented before the completion of puberty and 5 patients had received human GH (0.25 IU/kg IM 3 times a week for a mean of 6 years) until epiphyseal fusion. All patients received physiologic replacement of thyroxine, corticotropin, or sex steroids as needed. A control group of 17 subjects age-matched to these 10 patients also was studied. The larger group of GHD adults was matched to a normal reference population studied with an identical scanner. BMD was measured at the lumbar spine (L2 - L4), the femoral neck, and at Ward's triangle (a region of the proximal femur consisting predominantly of trabecular bone). The coefficient of variation for BMD measurement is 1% at the lumbar spine and 2% at the femoral neck.

The 10 subjects with GHD identified during puberty had a longer duration of GHD than the other 50 subjects, and those who were treated with GH were taller than those who were not treated. The mean BMD in the 5 untreated subjects was significantly lower than that of the controls and that of the GH-treated subjects. The 50 subjects with adult-onset GHD had mean BMDs of $89.9 \pm 2.2\%$ (lumbar spine), $96.1 \pm 1.1\%$ (femoral neck), and $96.0 \pm 2.7\%$ (Ward's triangle) when compared with the reference population. A significant negative correlation was found between the duration of GHD in all subjects and BMD measured at the lumbar spine or Ward's triangle.

Editor's comment: These findings suggest that untreated GHD during puberty results in diminished BMD at adulthood and that there may be some reduction in BMD with GHD acquired during adulthood. However, it should be noted that the standard errors of the mean for BMD determinations in the large group of GHD adults are very close to the coefficients of variation for the BMD measurement at the 3 sites. Thus, although a significant negative correlation was shown between duration of GHD and BMD measured at 2 sites, the clinical significance of these findings is not clear.

We recently reviewed reports of diminished BMD in adult men with a history of constitutional delay of puberty and in adult men with treated GHD (GGH 1992;8(3):13). In the latter study, the adults with GHD were all diagnosed and treated prior to epiphyseal fusion. In the present study, Hyer et al reported findings in a large cohort of adults who acquired GHD as adults and show somewhat different findings. Their findings, however, lend further support to the hypotheses that the diminished BMD associated with pubertal delay is secondary to a relative GH insufficiency during early adolescence. In a recent report in the *Journal of Pediatrics* (1993;122:37-45), Saggese measured BMD in 26 children aged 6.5 to 10.7 years with isolated GHD and showed diminished BMD at the distal radius (corrected for chronologic stature and bone ages) that was significantly increased by 12 months of GH therapy. These findings suggest a need for a larger prospective study to describe the relationship between BMD and GH secretion.

Hyer SL, Rodin DA, Tobias JH, et al. *Arch Dis Child* 1992;67:1472-1474.

William L. Clarke, MD

Congenital Idiopathic Growth Hormone Deficiency Associated With Prenatal and Early Postnatal Growth Failure

The authors identified 52 infants with presumed congenital growth hormone deficiency (CGHD) in whom therapy with GH was initiated before 2 years of age. Seven infants had septo-optic dysplasia, and the remainder had idiopathic CGHD (although imaging studies of the CNS are not reported). Delivery was normal in 67%, assisted in 10%, and by cesarean section in 23%. Two thirds of these infants were males. Mean birth length (48.3 ± 2.8 cm) and mean birth weight (3.14 ± 0.61 kg) were below published normal data ($P < 0.05$); the mean birth length was more than 2 standard deviations (SD) below the mean normal birth length. Infants with idiopathic CGHD were relatively obese at birth. Serum GH concentrations (determined in random or stimulated specimens) were less than 5 ng/mL in 85% of infants and between 5 to 10 ng/mL in the remaining 15%. GH deficiency was isolated in 42% of infants. Postnatally, growth velocity was slow in infants with CGHD. Seventy percent of subjects measured at 6 months, and 91% of these measured again at 12 months, had reported lengths falling below 2 SD from the norm. The investigators concluded that GH deficiency may impair in utero and postnatal growth, and

that GH is an important factor for human fetal and infantile growth.

Gluckman PD, Gunn AJ, Wray A, et al. *J Pediatr* 1992;121:920-923.

Editor's comment: *There has been uncertainty about the role of GH in fetal and early postnatal growth. However, neonates with GH insensitivity (Laron syndrome) and with isolated GH deficiency due to abnormalities of the GH gene are short at birth and have poor postnatal growth. These observations, and the current data from a large group of infants with CGHD, indicate that GH is a growth factor for the human fetus and infant, although the mechanism(s) through which it exerts these growth-promoting effects (idiopathic growth factor 1, idiopathic growth factor 2, or other growth factor) is unknown. It is also of interest that 15% of infants with CGHD had serum GH concentrations between 5 to 10 ng/mL at diagnosis; however, the nonuniformity of collection and assay of GH specimens makes interpretation of this observation less certain.*

Allen W. Root

Final Height After Growth Hormone Therapy in Peripubertal Boys With a Subnormal Integrated Concentration of Growth Hormone

The aim of this study was to evaluate the effect of growth hormone (GH) treatment on final height in boys treated at a peripubertal age with idiopathic short stature and possibly reduced GH secretion, as measured by continuous 24-hour blood sampling.

The cohort included 28 males with idiopathic short stature, aged 10 to 16 years (mean, 12.65 ± 1.4 years), and a mean target height of -2.0 ± 0.7 standard deviation score (SDS). The parents of these boys were short also, with a mean height of -2.0 SDS. At the time of study, the boys' mean height was -3.2 ± 0.9 SDS for age. Their mean growth velocity was -2.6 ± 1.1 SDS, ie, a mean of 3.4 cm/y, well below a norm of 4.5 cm/y. All patients were born at term, with normal birth length and weight. They were free of chronic disease or dysmorphic syndromes. Their mean bone age, evaluated according to Greulich and Pyle, was more than 2 SD below chronological age. They had a GH response of greater than 10 ng/mL (mean, 17 ng/mL) after stimulation by insulin, arginine, or clonidine. In contrast, the 24-hour integrated plasma GH level, measured by continuous pump blood withdrawal and sampling every 30 minutes, was definitely subnormal, below 3.2 ng/mL (mean, 2.25 ± 0.6 ng/mL). Plasma insulin-like growth factor 1 (IGF-1) was in the low normal range.

The patients and their families were randomly offered GH therapy. Eleven (group B, treated) accepted and received GH 0.75 IU/kg/wk until final height was achieved. GH was administered in 3 weekly doses for the first 2 years and then in daily doses. Seventeen patients did not accept, and served as controls (group A, untreated).

Patients of both groups were followed every 3 months until completion of growth, which was defined by a height gain of less than 0.5 cm for 6 months and complete epiphyseal fusion on X-ray films of the hand. The clinical and hormonal characteristics of groups A and B were very similar, without any significant differences. The Bayley-Pinneau mean predicted heights were 161.8 ± 5.9 cm for group A and 162.1 ± 7.6 cm for group B.

Growth velocity (GV) in the treated group was significantly

greater than in the untreated group during the first 2 years of study. In contrast, during years 3, 4, and 5, GV was slightly less in treated patients than in untreated controls. Plasma IGF-1 rose in both groups. In the untreated group, IGF-1 rose from 9.5 ± 11.0 to 29.4 ± 15.0 nmol/L, demonstrating a rise related to puberty. In the treated group, IGF-1 increased sharply during the first 2 years, reaching mean values of 36.6 ± 15.3 nmol/L and 35.4 ± 8.3 nmol/L, which were significantly higher at this time than control values, but dropped to comparable levels as those of untreated boys after the end of the second year of treatment.

There was no difference between groups A and B in mean age at the onset of puberty, 13.1 ± 1.8 years and 13.3 ± 1.7 years, respectively. Patients receiving GH had a slightly better prepubertal height gain than controls: $8.7 \text{ cm} \pm 4.0$ vs 5.6 ± 2.0 cm (NS); however, the total height gain of treated patients during puberty was not improved: 19.2 ± 4.0 cm vs $19.0 \text{ cm} \pm 2.2$ cm in controls, and the duration puberty was slightly shorter than in controls: 3.2 ± 0.7 years vs 4.0 ± 0.8 years (NS).

Although mean Bayley-Pinneau predicted heights at the onset of the study was the same (-1.8 SDS) in the 2 groups, the final height of the treated group was -1.5 ± 0.6 SDS vs -2.7 ± 0.7 SDS in the untreated group. This difference was significant at $P < 0.04$.

The authors point out that: (1) the main effect of GH was observed during the first 2 years of treatment, while most patients were prepubertal; (2) very little height gain, if any, was obtained by continuing GH therapy after the onset of puberty; (3) the patients in this study received a GH dosage in the range of classic replacement therapy; (4) GH therapy, administered for several years to peripubertal short boys with so-called neurosecretory dysfunction did not improve their final stature beyond their target height and predicted height; and (5) the mean final height of untreated boys was significantly below their target height and predicted height.

Among the authors' conclusions is that short patients with subnormal integrated concentration of plasma GH, whose GH

response to stimulation tests falls within the normal range, may improve their growth velocity when treated with substitutive doses of GH for 1 to 2 years before the onset of puberty. However, the authors also cited the high cost of the treatment, the potential disappointment if expectations are not met, and the possibility of adverse psychologic effects as factors to be considered in contrast to the incremental low height gains achieved in this specific group.

Zadik Z, Mira U, Landau H. *Horm Res* 1992;37:150-155.

Editor's comment: This paper deserves particular attention since it compares the final height of controls with short patients treated from late prepubertal years up to the end of pubertal development. Some degree of GH deficiency could be inferred since they had a slow prepubertal GV and endocrine investigations had shown what has been described as neurosecretory dysfunction. The natural history of the untreated subjects showed that their puberty started at the usual age, with a height below -3 SD; that they had a normal or low-normal gain of height during puberty; and that they reached

a mean final height of -2.7 SD, clearly less than their genetic target height of -1.9 SD and prepubertal predicted height of -1.8 SD. On the other hand, the patients who received GH at usual replacement doses probably gained more than 3 cm in height as compared with the untreated boys, before onset of puberty. The treated boys started puberty with a height of -2.5 SD and reached a final height of -1.5 SD, slightly better than their target height and predicted height.

A long-term study, this work affords pertinent data about a subgroup of constitutionally short boys with a poor prepubertal growth rate and so-called neurosecretory dysfunction. It suggests that GH treatment could be useful before puberty but in a very limited range, and would not be effective after the onset of sexual development. Thus, any decision to initiate GH treatment in such cases has to take into consideration the expected gain — or non-loss — of final height, the burden of treatment, including the cost, the potential present gap between the expectations of constitutionally short children and their families, and the probable long-term outcome of therapy.

Jean-Claude Job, MD

Wilms' Tumor and Insulin-Like Growth Factor 2

Wilms' tumor is a pediatric malignancy thought to arise when multipotential kidney blastemal cells fail to differentiate after birth and instead continue to proliferate. The occurrence of both sporadic and hereditary forms of Wilms' tumor and the early age of onset of bilateral kidney tumors suggest that Wilms' tumors result when a predisposing germ-line mutation is accompanied by a second mutation or loss of heterozygosity at the disease locus. A potential tumor suppressor gene, *wt1*, was cloned in 1990 by analyzing deletions at chromosomal locus 11p13. These are associated with Wilms' tumors. The *wt1* gene encodes a zinc finger DNA binding protein, called WT1, and this has been found to behave as a transcriptional repressor. The biologic significance of DNA binding and transcriptional regulation by WT1 is underscored by the observation that small deletions and point mutations in the WT1 Zn²⁺ fingers that abolish this DNA binding have been detected in a number of Wilms' tumors, especially in tumors associated with the Denys-Drash syndrome.

The fetal mitogen insulin-like growth factor 2 (IGF-2) is overexpressed in Wilms' tumor. In addition, the overgrowth disorder Beckwith-Wiedemann syndrome, which is characterized by loss of the maternal copy of the IGF-2 gene, also is prone to Wilms' tumors. For these reasons, Drummond et al have examined the interaction between the suppressor protein WT1 and the IGF-2 gene. They found that WT1 binds to multiple sites in the promoter

region of the IGF-2 gene, and that it acts as a potent repressor of IGF-2 transcription in vivo. Thus, a molecular basis for the overexpression of IGF-2 in Wilms' tumor has been identified, and these experiments suggest that the *wt1* gene negatively regulates blastemal cell proliferation by limiting the production of a fetal growth factor in the developing vertebrate kidney.

Drummond IA, Madden SL, Rohwer-Nutter P, et al. Repression of the insulin-like growth factor II gene by the Wilms' tumor suppressor WT1. *Science* 1992;257:674-678.

Editor's comment: This paper provides further insight into the question of specific molecular mechanisms of growth control, ie, what prevents all cells in all tissues of the body from proliferating indefinitely? In addition, there are many indications that both Wilms' tumor and Beckwith-Wiedemann syndrome are imprinted disorders, ie, they will develop when inherited from a parent of the same sex but will not when inherited from a parent of the opposite sex. The IGF-2 gene has been shown to be imprinted in mice such that only the paternally inherited gene is expressed. Thus, these experiments provide further insight into mechanisms of genomic imprinting as well as overgrowth in cancer.

Judith G. Hall, MD

New Genes May Shed Light on Cell Growth Control

Two independent lines of work in molecular biology have now begun to converge: research on the cancer-causing oncogenes and research on the signaling pathways that carry messages telling cells to start—or stop—dividing. Cell biologists have found that the pathways that transmit growth signals into the cell contain the proteins made by several known oncogenes. However, the question has been what are the intermediaries between the growth factor receptors and the oncogenes that, when activated, signal the cell to divide and to keep dividing until the oncogene signal is turned off?

One of the most common oncogene proteins involved in this type of signaling is *ras*, which acts as a relay point to the nucleus for all

the growth factor receptors examined so far. When the *ras* protein is locked in a permanent "on" position by a mutation, various types of cancer may result. Researchers have begun to understand how *ras* works (Reddy BV, Khanna SN, Jena P. *Science* 1992;258:1640). They have found that *ras* is turned on only when it has bound to the nucleotide GTP (guanosine triphosphate). It is turned off again via reaction with another protein, called GAP (GTPase-activating protein), that stimulates the breakdown of GTP into GDP (guanosine diphosphate), which in turn inactivates *ras* and remains tightly bound to it.

It would be disastrous, however, if this inactivated form of *ras*

could not be reversibly reactivated. Thus, the need for exchange proteins has been postulated — proteins that would remove the GDP and free up the *ras* protein so that it could bind new, activating GTP molecules. Such “*ras* exchangers” have been known for some time in yeast and in the fruit fly, *Drosophila*. Recently, several groups have identified and cloned genes for exchange proteins in mammals. These have been shown to be highly specific, stimulating the release of GDP from *ras*, but not from 2 other members of the *ras* superfamily that have different functions and presumably their own exchangers. There is some evidence, however, that the *ras* exchangers also may serve as a link to exchangers for other oncogene proteins, such as *rho*.

Marx J. *Science* 1992;257:484-485.

Editor's comment: Researchers are gradually beginning to unravel the nature and function of the genes that are essential to

normal cell growth but that, when improperly regulated, lead to devastating disorders such as cancer and neurofibromatosis. The discovery of intermediate proteins in the signaling pathway between growth factor receptors, on the cell membrane, and growth effector proteins such as *ras*, provides a mechanism for the delicately balanced regulation of cell division and quiescence. In addition, the fact that these intermediary exchanger proteins may serve as links to yet other signaling pathways allows us to begin to see how the incredibly intricate cascade and feedback pathways of the cell work on molecular level. And, as *ras* scientist Frank McCormick of Onyx Pharmaceuticals observes in Marx's article, this new information “has all sorts of therapeutic possibilities.” Drugs that inhibit *ras* activation might be used, for example, to treat diseases such as cancer and neurofibromatosis, in which growth stimulatory pathways are excessively active.

Judith G. Hall, MD

Relationship Between Urinary and Serum Growth Hormone and Pubertal Status

This study of correlations involved 31 prepubertal and 29 pubertal subjects. Three different groups were studied: (1) 21 patients, aged 6.9 to 18.2 years (7 prepubertal, 14 pubertal) who had received cranial irradiation of 18 to 24 Gy for acute lymphoblastic leukemia; (2) 18 subjects aged 3.8 to 18.9 years, among whom 10 were normal siblings of the irradiated patients and 8 were normal subjects with genetic short stature (10 prepubertal and 8 pubertal); and (3) 12 boys investigated once or twice for constitutional delay of growth and puberty (CDGP) for a total of 21 studies, among which 14 had 4- to 6-mL testes and 7 had testes with a volume of 8 to 12 mL.

Growth hormone (GH) secretion was evaluated as a 24-hour profile, with sampling every 20 minutes in groups 1 and 2, and as an overnight 12-hour profile in group 3. Urine was collected concurrently with blood sampling. Serum GH was assayed by the immunoradiometric assay (IRMA) technique. Urine concentration of GH was measured by a 2-step IRMA on dialyzed urine, with a sensitivity of 0.8 pg/mL and interassay coefficients of variation of 6.6% to 8.4%. The results were expressed as nanograms of GH per gram of creatinine. Renal function was checked and considered normal in all study subjects, although some of those with a history of leukemia had at times received short courses of 1 or 2 aminoglycosides.

The results in prepubertal children ($n=17$) showed a close correlation between mean serum GH (integrated concentration) and urinary GH: $r=0.88$ in group 1, 0.84 in group 2, and 0.82 in group 1+2 with $P<0.001$. There also were significant correlations in prepubertal children between nanograms of urinary GH per grams of creatinine and both the maximal peak ($r=0.86$) and the mean pulse amplitude ($r=0.71$) of the serum GH profile.

In the pubertal children of groups 1 and 2, considered together ($n=22$) or separately, there was no such relationship: $r=-0.26$ (NS) for the mean GH; $r=-0.29$ (NS) for the peak; and $r=-0.34$ (NS) for the mean amplitude of GH peaks on the profile.

In the early pubertal boys (stage 2) investigated for CDGP, the correlation was highly significant between urinary GH and mean serum GH ($r=0.74$, $P<0.001$), but less significant with the mean amplitude of pulses ($r=0.4$, $P<0.05$) and not significant with the peak serum GH value.

The authors point out that although GH excreted in urine represents less than 0.002% of cumulative serum GH, the correlations found are very close in prepubertal children and rather good

at early puberty. This is in contrast with the lack of correlation in late pubertal subjects. They conclude that measuring urinary GH may be a test for screening GH secretion in children, but it is inappropriate from mid to late puberty. They also stress that the impact of physiologic and pathologic changes of renal function upon filtration and excretion of GH by kidneys needs further investigation before considering urinary GH measurements as a reliable tool.

Crowne EC, Wallace WHB, Shalet SM, et al. *Arch Dis Child* 1992;67:91-95.

Editor's comment: There is some contrast between the good correlations between urinary and serum GH found by the authors up to early puberty and their rather negative conclusions. Their discussion is extensive, including many previous studies on urinary GH, done with more or less similar methodologies, and possibly this is the main reason for concluding in this sense. Whatever the reasons for this contrast, my opinion is that after more than 5 years of extensive work and a great number of clinical studies, measurement of GH in urine has never been proven to be a reliable means for appreciating somatotrophic secretion in clinical situations or for longitudinal studies in physiology of growth.

Jean-Claude Job, MD

2nd Editor's comment: The answer to the question, “Can measurement of urinary GH be used to diagnose growth hormone deficiency?” remains elusive after 30 years of investigation. In 1963, Geller and Loh (*J Clin Endocrinol Metab* 1963;23:1107) first attempted to do this, as have many others. Confirmation that GH deficiency can be diagnosed by the reported techniques has been elusive. Interpretation of the data in the abstract above is similarly guarded. Fortunately, Dr. Margaret MacGillivray, who has had a long-term interest in this question, will be writing an article for GGH after reviewing the literature and conferring with the investigators currently working in this field. We look forward to her review and summary. Hopefully, Dr. MacGillivray will give us a broad perspective and a definitive answer to our question.

Robert M. Blizzard, MD

MEETINGS CALENDAR

September 6-8, 1993 Frontiers of Paed Neuroendocrinol, London, Eng. Info: Dr MO Savage. Tel: 44-71-601-8487; Fax: 44-71-601-8505.

September 12-15, 1993 Ann Mtg of the Eur Soc for Paed Research (ESPR), Edinburgh, Scot. Sci Info: Prof N McIntosh. Tel: 44-31-667-2617; Fax: 44-31-668-2605. Genl Info: ESPR '93, Edinburgh Conf Ctr. Tel: 44-31-449-5111; Fax: 44-31-451-3199.

September 23-25, 1993 Natl Mtg of the Italian Soc of Paed Endo and Diabetol, Bari, Italy. Info: Dr L Cavallo. Fax: 39-80-536-4450.

September 30-October 2, 1993 Intl Symp on Developmental Endocrinol, Geneva, Switzerland. Info: Profs PC Sizonenko/M Aubert. Tel: 41-22-3824-592; Fax: 41-22-3824-588.

October 28-31, 1993 Somatotrophic Axis & the Reproductive Process in Health and Disease, Baltimore, MD. Info: Dr BK Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

November 7-11, 1993 Wkshp on the Superfamily of Receptors for Growth Hormone, Prolactin, Erythropoietin & Cytokines, Haifa, Israel. Info: Dr Z Hochberg. Tel: 972-3-635-5038; Fax: 972-3-635-1103.*

November 7-11, 1993 Molecular Basis of Endo Diseases, Barcelona, Spain. Info: Dr C Pavia. Tel: 34-3-2804-000; Fax: 34-3-2033-959.*

November 12-17, 1993 45th Postgrad Assembly of the Amer Endo Soc, San Francisco, CA. Info: C Huck. Tel: 301-571-1802; Fax: 301-571-1869.

December 9-12, 1993 GHRH, GH, IGF-1: Basic and Clinical Advances, San Diego, CA. Info: Dr BK Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

February 6-10, 1994 3rd Intl Symp on Insulin-Like Growth Factors, Sydney, Australia. Sci Info: Dr R Baxter. Tel: 61-2-516-6111; Fax: 61-2-516-1273. Genl Info: E Loveridge. Tel: 61-2-956-8333; Fax: 61-2-956-5154.

June, 1994 7th Intl Congress of Auxology, Budapest, Hungary.*

June 1-4, 1994 1st Intl Mtg of the Growth Hormone Research Soc, Aarhus, Denmark. Info: Dr JS Christiansen/JOL Jorgensen. Tel: 45-86-1255-55/Ext 2084; Fax: 45-86-1378-25.*

June 15-18, 1994 76th Ann Mtg of the Amer Endo Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1802; Fax: 301-571-1869.

June 22-25, 1994 33rd Ann Mtg of the ESPE, Maastricht, The Netherlands. Info: Congrex Holland. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

June 30-July 3, 1994 2nd Intl Conf on Prader-Willi Syndrome, Cambridge, Eng. Info: Dr BM Laurence.*

October 29-November 3, 1994 46th Postgrad Assembly of the Amer Endo Soc, Toronto, Canada. Info: C Huck. Tel: 301-571-1802; Fax: 301-571-1869.

November 6-11, 1994 15th World Cong of the IDF, Kobe, Japan. Sci Info: Prof S Baba. Tel: 81-78-303-0055; Fax: 81-78-302-7303.

*Confirmations not received upon publication.

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GROWTH

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Clinical Significance of Urinary Growth Hormone Measurements

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In the past decade, renewed interest in the measurement of urinary growth hormone (GH) output has occurred. Reports of suboptimal spontaneous GH production in short children whose GH stimulation

tests were normal prompted this interest.^{1,2} These children were classified as having GH deficiency because of defective neuroregulation of GH secretion. Assessment of spontaneous GH production requires either serial blood sampling at 20-minute intervals for 12 to 24 hours or constant withdrawal of blood via an indwelling catheter.¹⁻³ The former approach gives information about GH pulse frequency and amplitude, as well as mean and pool GH concentrations. The latter also yields an integrated GH level but provides no information about GH pulses. Both procedures are uncomfortable, labor intensive, and inappropriate for small children. Recently, the diagnostic validity of these tests has been challenged because mean or pooled GH concentrations of healthy children matched for age and pubertal stage overlap with concentrations observed in short children. Since timed urine collections integrate GH output, it was reasoned that measurement of urinary GH excretion would reflect endogenous GH production and provide a safe alternative method to screen short children for GH deficiency.

ASSESSMENT METHODOLOGY

Since 1985, the assays that have been used to re-evaluate the usefulness of urinary GH measurements include either improved radioimmunoassay (RIA) techniques; newly developed, ultrasensitive enzyme

Table 1: Urinary GH Output in Normal Subjects

Study	Assay	Sensitivity of Assay	Age (y)	Growth Hormone/ Creatinine (ng/g)
Albini ¹⁶	RIA after 50x concentration	0.15 ng/mL	7-15	Mean 35.3 ± 2.6
Granada ¹⁹	RIA after concentration	4 pg/mL	5-12	Mean 33 ± 22
			11-16	Mean 48 ± 27
			Adult	Mean 9 ± 4.4
Hashida ²³	ELISA	1.2 pg/mL	<2	Range 78 to 113
			2-5	Range 19 to 51
			6-15	Range 7.8 to 25
			28-35	Range 1.1 to 5.2
Tanaka ²¹	ELISA	3 pg/mL	2-16	Mean 13 ± 11.2
Porquet ¹⁰	IRMA	0.4 pg/mL	3-10	Mean 10.7 ± 0.2

RIA = radioimmunoassay

ELISA = enzyme-linked immunosorbent assay

IRMA = immunoradiometric assay

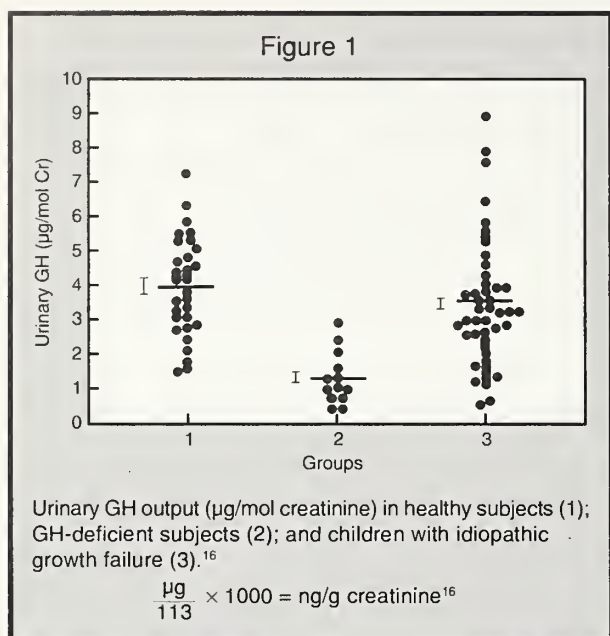
In This Issue

Contiguous Gene Syndromes..... pg 5

Results of the Diabetes Control and Complications Trials pg 11

Abstracts pg 12

Meetings Calendar pg 16



linked immunosorbent assays (ELISA); or immunoradiometric assays (IRMA).⁴⁻¹⁰ This article summarizes the information obtained from this research, and evaluates the utilization of urinary GH measurements for clinical and investigative purposes.

The authenticity of urinary GH has been confirmed by high-performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis, sephadex gel filtration, and double-monoclonal IRMA.^{5,10-12} The major form of GH in urine is a 22 kd peptide; the presence of a 20 kd form has been identified only in extensively concentrated urine.¹¹ Unfortunately, less than 0.001% of the GH secreted by the pituitary is excreted intact in urine. The remainder is degraded by the liver, kidneys, and peripheral tissues. Renal function has a profound effect on urinary GH levels. In healthy individuals, the fraction of GH in urine is the end product of glomerular filtration, subsequent reabsorption, and catabolism within tubular epithelial cells.

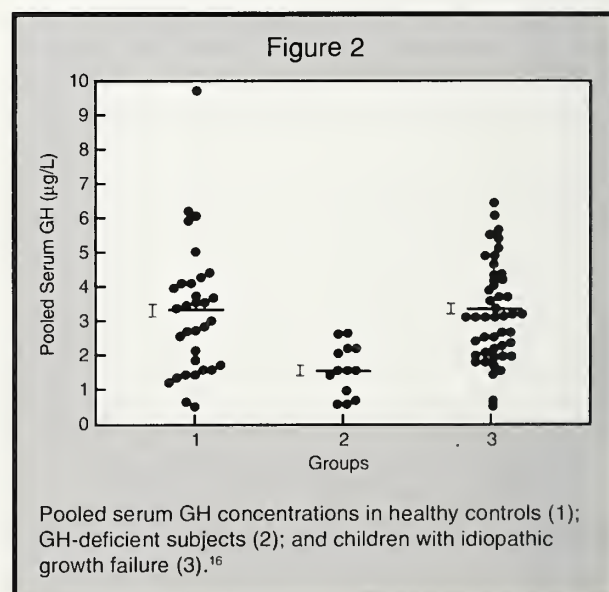
Prior to 1970, attempts to quantitate GH output in urine failed because of insensitive assays. In addition, the presence of interfering substances caused widely discrepant results and overestimations of GH excretion. In 1972, Hanssen improved the specificity and sensitivity of double-antibody GH RIA by first dialyzing and then concentrating the urine fifty-fold.⁴ These improvements minimized the problem of interfering substances, but the sensitivity was still limited to 0.15 to 0.3 ng/mL (150 to 300 pg/mL). Since 1985, significant gains have been made in developing ultrasensitive ELISA or IRMA assays that measure urine GH levels as low as 0.4 to 4 pg/mL. By using antibody-coated polystyrene beads to concentrate GH in urine, it is now possible

to omit prior dialysis and concentration steps for most samples. The recent development of a sensitive double-monoclonal IRMA has further increased the specificity of urine GH measurements.¹⁰

Disagreement exists as to the best method of standardizing urinary GH output in children of varying ages and sizes. The methods used include assessment of timed urine collections (ng/12 h or ng/24 h) based on chronologic age; weight (kg); surface area (M^2), and creatinine excretion.^{5,14-16} Since the amount of GH present in 24-hour urine collections from healthy subjects is extremely small, standardization on the basis of age, weight, or surface area results in considerable overlap of values between groups. The data are normalized most frequently on the basis of creatinine excretion. However, this approach may result in significant error because creatinine is dependent on lean body mass, which changes with age and clinical state.¹⁷ For example, the reduced lean body mass of hypopituitary children or elderly individuals is reflected in lower excretion of urinary creatinine, which falsely elevates estimates of urinary GH based on creatinine.¹⁶⁻¹⁸ Conversely, puberty is associated with an increase in lean body mass and creatinine excretion, which leads to blunting of the pubertal rise in urinary GH. Due to these uncertainties, some investigators have reported the absolute outputs of urinary GH in nanograms per time interval without standardizing for weight or creatinine.¹⁵⁻¹⁹

URINARY AND PLASMA GH CONCENTRATIONS

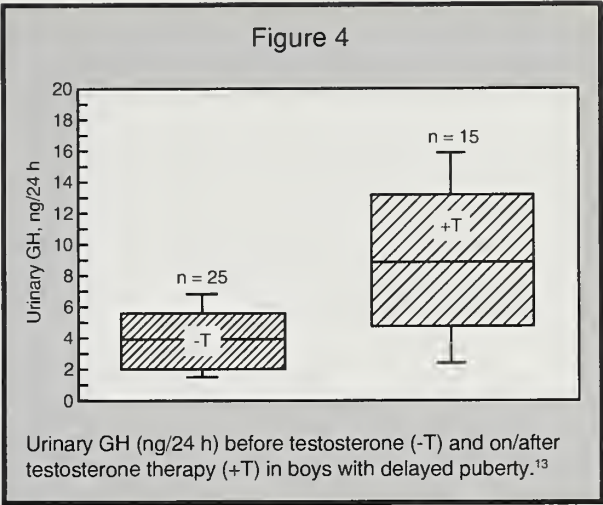
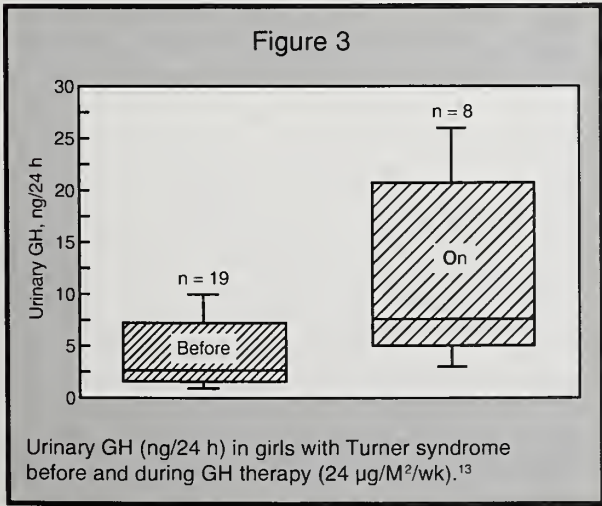
Urinary GH excretion correlates positively with plasma GH concentrations in most studies (mean or pooled GH levels and peak GH concentrations after growth



hormone-releasing hormone [GHRH] or stimulation tests). Also, the GH content of first morning urine specimens may correlate positively with 12- or 24-hour urine collections.^{14,16,20-22} A positive correlation was seen in prepubertal and early pubertal children when plasma and urinary GH levels were computed throughout puberty, as reported by Crowne et al.²² However, this correlation was not seen in subjects with advanced puberty. Although most investigators report a close relationship between urinary GH output and plasma GH levels, measurement of urinary GH excretion cannot be used to calculate pituitary GH production because the amount of intact hormone in urine is such a tiny fraction of the quantity produced. Therefore, small differences in excretion of urinary GH may produce large differences in calculation of the secretion of pituitary GH. Furthermore, widespread metabolism of GH in the body, coupled with renal processing of GH, undermines the validity of estimated GH production.

Girard¹³ recently reported that urinary GH output did not correlate with the dose of GH given to hypopituitary children, supporting evidence that the hormone undergoes extensive changes prior to appearance in urine.

In healthy children and adolescents, the mean output of urinary GH varies between 7.8 and 48 ng/g of creatinine (Table 1, page 1). Higher estimates of urine GH excretion were reported in studies utilizing RIA techniques than in those using ELISA or IRMA methods. The lack of agreement may be due to differences in the antibodies used or to matrix factors in the RIA method, since higher concentrations of urine were required. Alternatively, it is possible that omission of the concentration step prior to assaying by the ELISA or IRMA methods may have resulted in an inability to accurately measure the minute quantities of GH in dilute urines.^{7,9,15,16,19,21,23}



DISCUSSION

Many investigators have concluded that measurement of urinary GH output is not a valid screening test for GH deficiency in children who are failing to grow but who have normal GH responses to standard stimulation tests.^{16,21} Studies demonstrate that the overlap of urinary GH values between normal children and short subjects is similar to that observed for pooled serum GH levels in these populations (Figures 1 and 2). Consequently, neither urinary GH measurements nor serial serum GH studies appear to give reliable diagnostic information about the status of short "GH sufficient" children.^{16,24,25}

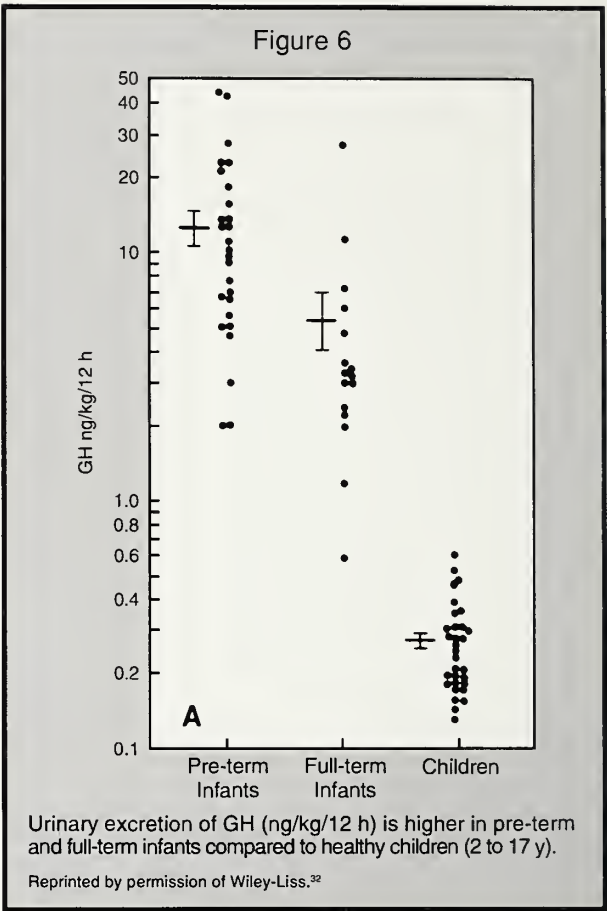
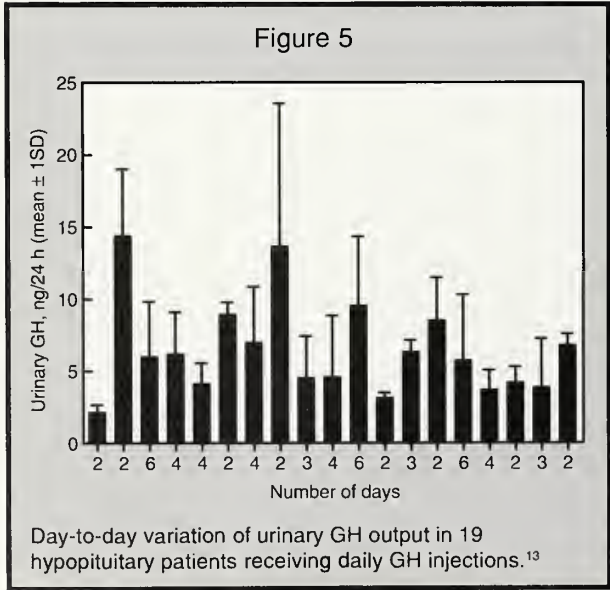
Urinary GH output will identify children with severe GH deficiency, as well as subjects who have GH excess.^{5,7} In the former group, standard GH stimulation tests are still needed for confirmation of GH deficiency. In acromegalic subjects, urinary GH output and plasma GH levels appear to yield similar diagnostic information at baseline and following therapeutic intervention. Therefore, urinary GH measurements may be of little additional clinical value in acromegaly.

Urinary GH measurements also have been informative in studying physiology. Estimates of urinary GH output have been used to monitor the effects of treatment with GH or testosterone.¹³ Not surprisingly, hypopituitary children treated with GH have increased outputs of GH compared to the low values observed in the baseline periods. Also of note, girls with Turner syndrome who received higher than standard doses of GH exhibit greater outputs of urinary GH than treated hypopituitary children (Figure 3). Following testosterone treatment (50 to 100 mg/mo), boys with delayed adolescence excrete at least twice as much GH in urine as compared to pretreatment values (Figure 4). The latter observation confirms

that androgen-stimulated GH secretion is reflected in urine. The output of urinary GH in testosterone-treated boys has been shown to be similar to the amount observed in girls with Turner syndrome who received higher doses of GH. Urinary GH measurements have also been used to monitor the effects of dose and frequency of GH injections in GH-deficient children. However, considerable day-to-day variability has been observed in hypopituitary subjects receiving daily GH therapy (Figure 5).^{13,26}

Urinary GH measurements have increased our knowledge about the relationship between the output of insulin-like growth factor 1 (IGF-1) and GH in urine. These levels are positively correlated. Hypopituitary children treated with GH show a prompt rise in both urinary IGF-1 and GH excretion. This observation confirms that renal excretion of IGF-1 is GH-dependent.²⁷

Measurement of urinary GH output has provided qualitative information about the relative output of GH from infancy through adulthood (Figure 6 and Figure 7). The data suggest that more GH is excreted in urine during infancy than in childhood or adulthood.^{23,28,29} A similar pattern of IGF-1 excretion also is observed in these age groups. During early childhood, the positive relationship evidenced between GH and IGF-1 in urine differs from the relationship seen in plasma, since plasma IGF-1 levels are low during this period of rapid somatic growth. Opinions differ as to the interpretation of urinary GH data in pubertal children. When the data are standardized for weight or creatinine, the output of urinary GH does not increase in puberty.^{5,19} A pubertal rise in urinary GH output is seen only when the data are not standardized (ie, expressed as nanograms per 12 or 24 hours).^{15,19}



Abnormalities of glomerular or tubular function interfere with the reliability of GH measurements in urine. Urinary GH correlates negatively with creatinine clearance and positively with β_2 -microglobulin and albumin excretion.^{30,31} Thus, urinary estimates of GH excretion are inaccurate in children with renal insufficiency or diabetic nephropathy.

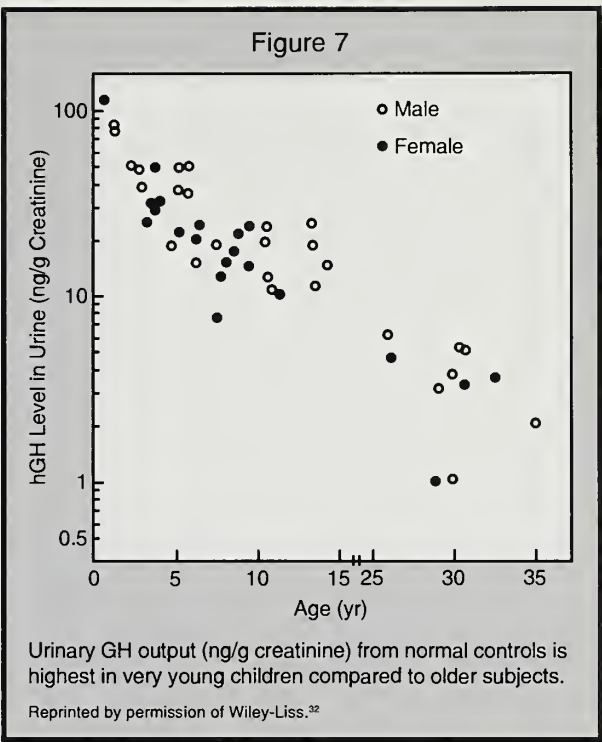
CONCLUSIONS

Measurement of urinary GH excretion is not a valid screening test for GH deficiency in short children who have normal GH stimulation tests. This method will identify the child with severe GH deficiency and the child with GH excess, but confirmation by serum GH measurements is still needed. Furthermore, assessment of urinary GH provides no information about GH pulsatility and cannot be used in patients with abnormal renal function. Since urinary testing is noninvasive, repetitive measurements of urinary GH can be used to gather information about relative outputs of GH over time in small children and to assess changes in urinary GH excretion before, during, and after therapeutic interventions aimed at increasing or decreasing GH production. Also, measurements of urinary GH and IGF-1 suggest

that a closer relationship exists for these peptides in urine than in plasma for all ages. To date, however, there is insufficient evidence in the literature to prompt this author to recommend measurement of GH in urine except as a possible research technique. Even in the latter instance, caution in interpretation of the data is mandatory, because only trends in production — rather than production rates — can be evaluated.

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Contiguous Gene Syndromes

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In 1986, Schmickel¹ proposed the term contiguous gene syndromes for a group of disorders characterized by microdeletions or microduplications of chromosomal segments associated with clusters of single gene disorders. These disorders were recognized clinically prior to their cytogenetic localization, distinguishing them from the classic group of deletions or duplications recognized cytogenetically prior to, or concomitantly with, their clinical delineation (eg, Wolf-Hirschhorn syndrome, cri du chat syndrome, 18p deletion, and 18q deletion). Regardless, these classic chromosomal deletion or duplication syndromes may yet be revealed as contiguous gene

syndromes once the size and extent of the critical region on the chromosome (as determined by molecular analysis) has been correlated with the clinical phenotype.

Schmickel proposed 2 types of contiguous gene syndromes — those with and those without visible cytogenetic abnormalities.¹ Both types of cytogenetic abnormalities have been described for the same disorder, so that in fact, there is probably a phenotypic spectrum for each specific disorder relating to the size and location of the deletion. Schmickel described 7 autosomal and 1 X-linked contiguous gene syndromes. Since his paper, most of the contiguous gene syndromes described have involved microdeletions. This may be due to the fact that the phenotypic effects resulting from microdeletions are more obvious clinically than those associated with microduplications. As outlined by Ledbetter and Cavenee² and by Schinzel,³ microdeletion and microduplication syndromes have several features in common (Table 1, page 6).

The identification and delineation of contiguous gene syndromes has been evolving. The ability to study these conditions is dependent on 3 components:

- (1) The identification and clinical evaluation of patients with suspected contiguous gene syndromes. Most patients with these syndromes display varying degrees of mental retardation; thus, one might suspect a contiguous gene syndrome in a mentally retarded individual with one — or multiple — mendelian traits that are not usually associated with mental retardation. Chromosome analyses should be performed to search for cytogenetic abnormalities that may lead to further delineation of the disorder.
- (2) Access to high-quality cytogenetics. The newly recognized contiguous gene syndromes were initially detected by routine metaphase chromosome analyses, which identified obvious structural chromosome abnormalities (eg, translocations, ring chromosomes). High-resolution analyses of over 500 bands have then been used to characterize the critical chromosome regions of a specific syndrome.² More recently, the isolation of molecular probes from these critical regions coupled with the use of fluorescent in situ hybridization (FISH) techniques, has facilitated the detection of deletions along chromosomes that previously appeared normal via high-resolution analyses, in disorders such as Prader-Willi/Angelman syndrome, Miller-Dieker syndrome, and DiGeorge syndrome (DGS).⁴⁻⁷ FISH is an important adjunct for studying disorders mapped to telomeric regions of chromosomes that have been associated with subtle or cryptic translocations inherited from one parent^{6,8} (eg, Miller-Dieker syndrome, Wolf-Hirschhorn syndrome, and cri du chat syndrome). Cytogenetic studies of both parents using FISH may be beneficial for determining the risk for recurrence in subsequent offspring and as a means of prenatal diagnosis in families where inheritance of such a disorder has already occurred. Based on the higher sensitivity and specificity of FISH, this technology may displace high-resolution chromosome analyses in the diagnosis of many of the contiguous gene syndromes.
- (3) The mapping of critical chromosome regions can be further enhanced by employing standard molecular techniques, including Southern blot analysis and polymerase chain reaction.²

CLASSIC MICRODELETION SYNDROMES

Langer-Giedion syndrome (LGS) was first described in 1969.⁹ The clinical features of LGS include sparse scalp hair, bulbous or pear-shaped nose, cone-shaped phalangeal epiphyses, and multiple cartilaginous exostoses. The majority of

patients are mentally retarded, although there is quite a range in the degree of retardation such that some patients have been reported to have normal intelligence.¹⁰ Prior to the distinction of LGS a similar disorder, **trichorhinophalangeal (TRP) syndrome**, was described by Giedion in 1966.¹¹ Patients with TRP are clinically similar to those with LGS (also known as TRP2) except for the absence of multiple cartilaginous exostoses and mental retardation. Beginning in 1980, several patients with LGS were described with de novo deletions of 8q24.1. Subsequent studies have identified deletions of 8q24.1 in about 50% of patients.¹² It has been proposed that the critical regions coding for TRP1 and multiple exostoses (ME; a condition that is also an isolated autosomal dominant disorder) are located in close proximity along this region of 8q.¹³ Although it now appears that both TRP1 and LGS (or TRP2) are mapped to this region, linkage studies of families with ME have not demonstrated linkage to chromosome 8 in all families.¹³ Based upon these findings, ME may be a heterogeneous condition localized in the critical region of 8q24.

The association of **aniridia and Wilms tumor** was first described in 1964.¹⁴ Subsequently in 1978, a deletion of chromosome band 11p13 was identified in patients with a complex of abnormalities,

Table 1
**Features of Microdeletion/
Microduplication Syndromes**

1. The syndromes were recognized prior to knowing the cytogenetic etiology, although occasionally chromosomal abnormalities have been reported.
2. Usually these syndromes are sporadic, but occasionally they are familial or dominant.
3. Cytogenetic abnormalities are usually detectable by high-resolution chromosome analysis. Studies using fluorescent in situ hybridization (FISH) may become more practical.
4. Not all patients have visible cytogenetic abnormalities, although the frequency may increase with the use of FISH. However, some patients may have submicroscopic molecular deletions.
5. Specific features of the syndrome may occur as single mendelian traits.
6. Multiple, unrelated loci that are physically contiguous in the critical region are thought to be involved. The patient's phenotype frequently correlates with the deletion or duplication of these contiguous genes.

Adapted from Ledbetter and Cavenee² and Schinzel.³



Figure 1: Critical Chromosome Regions

The critical chromosome regions for the syndromes presented herein are designated on the ideogram. The critical regions for these disorders are designated on the left side of the chromosome for deletions and on the right side for duplications as follows:

Region

- AHD – Arteriohepatic dysplasia (Alagille syndrome) (del 20p11.23p12.2)
- ATMR – Hemoglobin H/ α -thalassemia-mental retardation (del 16p13.3)
- BWS – Beckwith-Wiedemann syndrome (dup 11p15)
- CDCR – Choroideremia, deafness, clefting, retardation (del Xq21)
- CES – Cat-eye syndrome (dup 22q11)
- CMT1A – Charcot-Marie-Tooth disease, type 1A (dup 17p11.2p12)
- DGS – DiGeorge/Velocardiofacial syndrome (del 22q11)
- DMD – Duchenne's muscular dystrophy and contiguous genes (del Xp21)
- GCPS – Greig cephalopolysyndactyly (del 7p13)
- HOLO – Holoprosencephaly (one form) (del 7q34)
- KAL – Kallmann syndrome and contiguous genes (del Xp22.3)
- MDS – Miller-Dieker syndrome (del 17p13)
- PWS/AS – Prader-Willi/Angelman syndrome (del 15q12)
- RB – Retinoblastoma (del 13q14.11)
- RTS – Rubinstein-Taybi syndrome (del 16p13.3)
- SMS – Smith-Magenis syndrome (del 17p11.2)
- TRP – Trichorhinophalangeal/Langer-Giedion syndrome (del 8q24.1)
- WAGR – Wilms tumor, aniridia, genital abnormalities, retardation (del 11p13)

including Wilms tumor, aniridia, genital abnormalities, and mental retardation (WAGR).¹⁵ A number of genes have now been mapped to this region, including catalase and the beta subunit of follicle-stimulating hormone (FSH β), in addition to the Wilms tumor gene (WT1) and aniridia (AN2).¹⁶

Drash syndrome, comprised of the triad of nephropathy, Wilms tumor, and genital abnormalities, may, at least in some cases, also be associated with the WAGR microdeletion syndrome.¹⁷ In addition, the Wilms tumor appears to be associated with acquired homozygosity of WT1 and preferential nonrandom loss of the maternal allele, suggesting an imprinting effect.¹⁸ Lastly, WT1 has also been implicated in abnormalities of renal development, suggesting that the known spectrum of this microdeletion syndrome may be expanded with future research.¹⁹

In many respects, the association of **retinoblastoma (RB) and deletion of chromosome 13q14.11** was one of the first microdeletion syndromes to be described and one of the first genes to be cloned.^{20,21} In addition to RB, children with visible deletions may have mental retardation and facial dysmorphism, including broad nasal bridge, upturned nares, elongated philtrum, and thin upper lip.²² The tumor appears to arise by reduction to homozygosity of the RB locus.²¹

Prader-Willi syndrome (PWS), first described in 1956, consists of neonatal hypotonia, feeding difficulty, and genital hypoplasia. Hyperphagia and obesity develop during the first 1 to 2 years of life.²³ Other characteristics associated with PWS include distinctive facial features, short stature, small hands and feet, and hypopigmentation. In 1981, Ledbetter

et al²⁴ described a small interstitial deletion of chromosome 15 between bands q11 and q13 found in about half of PWS patients. In 1987, another disorder, **Angelman syndrome (AS)**, was also discovered to have a similar deletion of the same region of chromosome 15.²⁵ Angelman syndrome is characterized by microcephaly, macrosomia with prominent tongue, hypotonia, ataxic gait, excessive laughter, seizures, and severe mental retardation; some patients also have hypopigmentation.²⁶ PWS and AS have now been shown to be the prototypes of genomic imprinting in humans.²⁷ The majority of PWS individuals have deletions of paternal origin. Thirty percent of PWS patients with no deletion have been found to have maternal disomy of chromosome 15, usually heterodisomy, with no paternal chromosome 15 contribution. This may arise by maternal nondisjunction, resulting in a trisomy 15 conceptus with loss of paternal chromosome 15. In addition, a small nuclear ribonucleoprotein (snRNP) was identified as a maternally imprinted candidate gene for PWS.²⁸ Conversely, AS has been found to be associated with maternally derived deletions of chromosome 15 in over half of the cases. However, only 3% to 5% of cases have been shown to have paternal disomy of chromosome 15, primarily isodisomy.²⁹ The remaining AS patients have biparental inheritance, with a copy of chromosome 15 from each parent and no cytogenetic or molecular deletion.²⁹ Several families with multiple affected individuals have now been described with AS but without cytogenetic deletions; in each case the same chromosome 15 has been maternally inherited, suggesting paternally imprinted mutations of the hypothetical

AS gene.³⁰ Recently, the mouse pink-eyed deletion gene has been mapped to the PWS/AS critical region. This deletion gene may be involved in the hypopigmentation seen in both disorders as well as oculocutaneous albinism type II.³⁰

Miller-Dieker syndrome is a rare malformation syndrome consisting of type I lissencephaly and dysmorphic facies, resulting from a deletion of chromosome 17p13.3, initially described in 1983.³² Visible deletions of 17p13.3 have been identified in about 50% of patients.³³ In patients without visible deletions, large molecular deletions have been detected. The use of FISH has enhanced detection of chromosome 17 deletions associated with Miller-Dieker syndrome.⁶ In addition, probes isolated from the Miller-Dieker critical region have identified molecular deletions of 17p13.3 in a small percentage of patients with isolated lissencephaly.³⁴

DiGeorge syndrome (DGS), first described by DiGeorge in 1965, is characterized by a group of defects resulting from abnormalities in the development of the third and fourth branchial arches, producing thymic hypoplasia, parathyroid hypoplasia, and conotruncal cardiac defects, in addition to facial dysmorphism.³⁵ The majority of DGS patients initially come to a physician's attention due to congenital heart defects; a small percentage initially present with manifestations of hypocalcemia.³⁵ Although the pathogenesis of DGS appears to be fairly consistent, (ie, maldevelopment of the branchial arches), the etiology is heterogeneous.³⁶ Most cases of DGS are sporadic, although familial cases have been known. Prior to the use of molecular techniques, about 15% of cases were shown to have cytogenetic abnormalities, primarily deletions of 22q11³⁷; however, other chromosome abnormalities, including deletion of 22q11, also have been described.³⁷ In addition, DGS has been associated with teratogenic

exposure, including alcohol, isotretinoin, and maternal diabetes.³⁶ It has also been observed in conjunction with other genetic disorders, including **velocardiofacial syndrome** (VCFS), **Zellweger syndrome**, and **Kallmann syndrome**.³⁶ Recent studies have confirmed the presence of microdeletions of chromosome 22q11 in most patients with DGS. Cytogenetically visible deletions are apparent in as many as 30% of DGS cases, and molecular deletions are detectable in 90% of cases.^{38,39}

Velocardiofacial syndrome (VCFS, or **Sprintzen syndrome**) is characterized by palatal defects, including cleft palate; hypoplastic alae nasi with a long nose; learning disorders or mental retardation; and congenital heart disease, primarily conotruncal defects. It is inherited in an autosomal dominant fashion⁴⁰ with as many as 90% of VCFS patients having visible deletions of 22q11. In about 90% of these patients, the 22q11 deletions have been shown by FISH or molecular analysis.^{41,42} Lastly, although some patients with CHARGE association (ventricular septal defect, vertebral anomalies, anal atresia, tracheoesophageal fistula, radial and renal anomalies) also have DGS-type anomalies, it appears that only a small percentage of CHARGE patients have deletions of chromosome 22 demonstrable by cytogenetic or molecular techniques.⁴¹

RECENTLY DESCRIBED MICRODELETION AND MICRODUPLICATION SYNDROMES

Microdeletion Syndromes

As of 1986, one contiguous gene deletion syndrome on the X chromosome had been described. This involved **deletion of Xp21**, which included the genes for Duchenne's muscular dystrophy, chronic granulomatous disease, the McLeod phenotype, retinitis pigmentosa, glycerol kinase deficiency, adrenal hypoplasia, and ornithine transcarbamoylase deficiency.⁴³ More recently, 2 new contiguous gene deletion syndromes of the X chromosome have been described. The first — a **deletion of Xp22.3** — is characterized by X-linked ichthyosis with steroid sulfatase deficiency, Kallmann syndrome, chondrodysplasia punctata, mental retardation, short stature, ocular albinism, and may be expanded to include Aicardi syndrome and Goltz syndrome.⁴⁴ The second is a **deletion of Xq21** that is associated with choroideremia, deafness, cleft lip and palate, and mental retardation.⁴⁵

A number of new contiguous gene/microdeletion syndromes have been described since Schmickel's paper was published in 1986. **Greig cephalopolysyndactyly syndrome** (comprised of craniosynostosis associated with polysyndactyly and occasionally with mental retardation) was found to be

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associated with translocations or deletions of chromosome 7p13, suggesting that this may be a contiguous gene deletion syndrome.⁴⁶

Muencke and colleagues recently have shown association of holoprosencephaly and associated features in patients with different sized deletions of chromosome 7q34.⁴⁷ The features appear to vary with the size and extent of the deletion.

The association of α -thalassemia with mental retardation has been linked to deletions and subtle cryptic translocations involving chromosome 16p13.3.⁴⁸ In a group of 8 patients, 4 were found to have unbalanced chromosome translocations and the remainder had deletions of 16p13.3 as detected by high-resolution cytogenetic or molecular analysis, including FISH.⁴⁹ However, there is also an X-linked form of this disorder.⁵⁰

Rubinstein-Taybi syndrome (RTS) is phenotypically characterized by dysmorphic facial features, including a beaked nose, prominent columella, hypoplastic maxilla, and down-slanted palpebral fissures; broad thumbs and first toes; and varying degrees of mental retardation.⁵¹ After previous association of RTS with chromosomal abnormalities involving 16p13, submicroscopic deletions were identified.⁵² In 6 of 24 patients with normal appearing chromosomes, a submicroscopic deletion was detected by FISH.⁵² Since at least 1 gene for tuberous sclerosis and adult polycystic kidney disease gene are also located in this region, it will be of interest to determine whether these disorders may also be part of a contiguous gene deletion syndrome with RTS.

Smith-Magenis syndrome (SMS), with deletion of chromosome 17p11.2, was initially described in 1982.⁵³ The disorder consists of brachycephaly, midface hypoplasia, broad nasal bridge, prominent jaw, short broad hands, speech delayed, varying degrees of mental retardation and behavioral abnormalities, including onychotillomania (compulsive picking at the nails) and polyembolokoilomania (insertion of foreign bodies into orifices).⁵⁴ The disorder may be relatively common; more than 100 patients have been described over a relatively short period. Approximately two thirds of patients have evidence of a peripheral neuropathy associated with normal nerve conduction velocities.⁵⁴ Although the gene for **Charcot-Marie-Tooth** disease type 1A (CMT1A) is usually associated with a duplication within 17p11.2p12,⁵⁵ the CMT1A region does not appear to be deleted in the majority of SMS patients, including those with peripheral neuropathy.⁵⁶ Thus, the etiology of peripheral neuropathy in SMS patients is still unclear. In addition, about two thirds of patients with SMS have sleep disorders; 2 patients have been shown to have absence of REM sleep on

sleep study.⁵⁴ A number of other patients have demonstrated reduced amounts of REM sleep. Thus, it has been hypothesized that a gene involving REM sleep may be localized to this region. Though in some ways, SMS fits the criteria for a microdeletion syndrome, additional work is needed before it can truly be called a contiguous gene deletion syndrome. While the majority of patients have visible deletions of chromosome 17 revealed by high-resolution cytogenetics, studies of several patients with clinical features of SMS and whose chromosomes appear normal are currently ongoing to detect submicroscopic deletions of this region.

Arteriohepatic dysplasia (AHD), or Alagille syndrome, is an autosomal dominant disorder associated with chronic cholestasis and paucity of interlobular bile ducts, dysmorphic facies, posterior embryotoxon, butterfly-like vertebral arch defects, and peripheral pulmonic stenosis or hypoplasia.⁵⁷ A recent report by Schnittger et al⁵⁸ showed 9 patients with visible deletions of chromosome 20p11.23p12.2.

Microduplication Syndromes

Beckwith-Wiedemann syndrome (BWS) consists of macrosomia, macroglossia, omphalocele, hypoglycemia due to pancreatic islet cell hyperplasia, transverse earlobe creases, hemihypertrophy, and advanced bone age.⁵⁹ Individuals with BWS have an increased risk of malignancy, particularly Wilms' tumor, adrenocortical carcinoma, and hepatoblastoma. A small percentage of patients with BWS have been observed to have duplications of chromosome 11p15.⁶⁰ However, the majority of BWS patients do not have a visible chromosome 11 abnormality.⁵⁹ More recent studies have suggested that parental imprinting may play a significant role in BWS. It appears that the duplication 11p15 in BWS patients is primarily of paternal origin; a few of the patients without cytogenetic duplications have paternal uniparental disomy for markers in the 11p15 region.⁶¹ In addition, there are several cases of maternal inheritance of BWS with an inversion or translocation of chromosome 11 in band p15.5. Thus, in the strict sense, BWS is not a true microduplication syndrome, at least in most cases.

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Duplications of chromosome 17p11.2p12 have been described.⁶² The clinical features of patients with this disorder have been variable, although the majority have hypotonia, decreased reflexes, and club foot. With the finding of duplication of a small region within 17p11.2p12 in association with CMT1A,⁵⁵ a number of patients with cytogenetic duplications of this region were studied to determine whether nerve conduction findings consistent with CMT1A would be present. Patients who have duplication for PMP22, the candidate gene for CMT1A,⁶³ also have findings consistent with CMT1A, while those patients without this PMP22 duplication do not have features of CMT1A.⁶² These duplications have been visible without high-resolution cytogenetics. At least one patient with the duplication of 17p11.2p12 and CMT1A also has absence of REM sleep, lending support to the idea that a gene involved with sleep regulation is also located within this chromosomal region.

Another possible contiguous gene duplication syndrome is the **cat-eye syndrome**, which is due to duplication of chromosome 22q and consists of coloboma of the iris and anal atresia.⁶⁴ In addition, many of these patients have ear abnormalities and cardiac defects. Analysis of the duplicated region in these patients is currently being studied by McDermid and colleagues.⁶⁵

FUTURE ISSUES

Advances in molecular genetics and cytogenetics, and the subsequent increase in the number of single gene disorders mapped to specific chromosomal regions, will undoubtedly lead to the delineation of additional contiguous gene syndromes. Several syndromes (ie, de Lange syndrome⁶⁶), are currently under investigation, and others that have been recently mapped may lead to the identification of contiguous gene syndromes located within the same critical region.

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Results of the Diabetes Control and Complications Trials

Reviewed by William L. Clarke, MD

The Annual Scientific Session of the American Diabetes Association was held in Las Vegas, June 12 through 15, 1993. The highlight of this meeting was the report from the Diabetes Control and Complications Trial (DCCT), presented to the largest gathering (more than 6,000 individuals) ever to be assembled in the 53-year history of the American Diabetes Association. The DCCT is a randomized prospective study of the hypothesis that intensive treatment of insulin-dependent diabetes that reduces hyperglycemia will delay the onset and/or reduce the progression of microvascular complications. More than 1,400 subjects aged 13 to 39 years participated in the trial. There were 2 distinct cohorts: (1) a primary prevention cohort comprised of individuals with diabetes of 1 to 5 years' duration and with no background retinopathy; and (2) a secondary intervention cohort comprised of individuals with diabetes of 1 to 15 years' duration and with retinopathy of minimal to moderate degree. Eighteen percent of the primary cohort were adolescents, while 10% of the secondary cohort were adolescents.

Subjects were randomized in each group to an intensive treatment or conventional treatment group. The goal in the conventional treatment groups was to prevent symptoms of hypoglycemia or hyperglycemia. They were treated with 1 to 2 daily insulin injections, daily self-monitoring, quarterly glycosylated hemoglobin determinations, diet and exercise education, and quarterly physician visits. Glycosylated hemoglobin values were not reported to the physician or patient unless they exceeded 2 standard deviations (SD) above the mean for this treatment group. Self blood glucose monitoring was not used for daily treatment management. The goal for the intensive treatment groups was to have pre-meal blood glucose values between 70 and 120 mg/dL; post-meal glucose levels <180 mg/dL; 3:00 AM blood glucose levels >65 mg/dL; and glycosylated hemoglobin levels at the upper limit of the normal range. These groups were treated with 3 or more daily insulin injections or with continuous subcutaneous insulin infusion pumps. Blood glucose was measured 4 or more times per day, and abnormal values prompted frequent changes in insulin, diet, and exercise. Monthly clinic visits were required.

Ninety-nine percent of individuals from both cohorts completed the entire study. Retinal and renal

data were collected on more than 98% of patients and neurologic evaluations were available for more than 97%. Although the intensive treatment groups did not achieve the goal of maintaining glycosylated hemoglobin values at the upper range of normal, there was always a consistent 1% to 2% difference between their mean levels and those of the conventional treatment groups. Adolescents consistently had glycosylated hemoglobin values approximately 1% above the average for nonadolescents. Capillary blood glucose levels were measured quarterly before and 90 minutes after each meal, and averaged across subjects. Mean blood glucose averaged 155 mg/dL in the intensive treatment groups and 231 mg/dL in the conventionally treated groups.

Retinal evaluations included stereoscopic photographs of the fundi, which were evaluated by blinded readers. Retinal findings were similar in both groups in the primary intervention cohort (background retinopathy at time zero) for the first 3 years; however, by the end of the study, a 76% reduction in retinopathy was seen in the intensive treatment group compared with the conventional treatment group. In the secondary intervention cohort (background retinopathy at time zero), progression of retinopathy in the intensive treatment group was reduced by 34% compared with the conventional treatment group. All subgroups, including adolescents, benefited from intensive insulin therapy.

Nephropathy was evaluated by albumin excretion. Microalbuminuria (albumin excretion rate [AER] between 40 and 300 mg/24 h) was reduced by 38% in the combined intensive treatment group as compared to the conventional treatment group. Clinical albuminuria (AER \geq 300 mg/24 h) was reduced by 56% and clinical nephropathy (AER \geq 300 mg/24 h

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and creatinine clearance <70 mL/min) was reduced by 60%. Neuropathy, measured by heart rate variation to deep breathing and postural changes and nerve conduction studies, was reduced by 60% in the intensive treatment group. There were no differences between the 2 groups with regards to the development of hypertension or elevated triglyceride levels, but low-density lipoprotein cholesterol was significantly lower in the intensive treatment groups. No differences were observed in neuro-behavioral or quality-of-life measures between the intensive and conventional treatment groups. Intensive therapy of insulin-dependent diabetes did not reduce quality of life or adversely affect neuro-behavioral or psychological parameters.

The complications of intensive therapy included: weight gain (an average 10 lbs more than in the conventional treatment groups); catheter infection; and severe hypoglycemia, for which the incidence was 3 times greater in the intensive treatment groups.

The investigators stated that the benefit ratio could be less favorable in patients with recurrent severe hypoglycemia or hypoglycemic unawareness; those with far advanced complications such as renal failure; patients with coronary artery or cerebral vascular

disease; or children less than 13 years of age (who were not included in the study).

The conclusion of this study is that the majority of insulin-dependent diabetic patients should be treated with intensive therapy with the expectation that the long-term outcome will be measurably improved. The study was not designed to determine the level of glucose control at which the prevention of complications could be maximized while minimizing the risk of severe hypoglycemia. However, the data clearly support the concept that any reduction in glucose level has potential benefits. The debate regarding the role of glucose control and the development of microvascular complications of insulin-dependent diabetes mellitus has been concluded.

Erratum

In **GROWTH, Genetics, & Hormones** Vol. 9, No. 2 (June 1993) an error on page 13 identifies growth-promoting mechanisms in Dr. Root's editorial comment as *idiopathic*-like growth factor 1 and 2. The correct description should have been *insulin*-like growth factor 1 and 2.

Abstracts From the Literature

Effects of Changes in Nutritional Conditions on Growth and on Timing of Puberty

Longitudinal studies of growth in different countries provide an index of the nutritional and hygienic status of their populations. Secular trends in growth and its relationship with socioeconomic levels are given by J.M. Tanner in a retrospective analysis.¹ Whatever the country, the era, and the general conditions of study, similar data were obtained, with increased height change observed between successive generations within specific ethnic societies and among socioeconomic strata within each ethnic group.

The preschool years, between 18 and 24 months and 3 and 4 years, seem the age period when different trends between contemporary classes, between generations, and between children of the developing and industrialized countries mainly appear. This is the period when the legs are growing faster relative to the trunk, and when larger trends for leg length than for sitting height are found. A current hypothesis includes not only nutrition but morbidity as a factor in the young to account for the social/secular trends. For example, growth patterns relate to the degree of catch-up growth occurring following successive episodes of infection, during which growth has slowed down. Restoration of the genetically endowed growth potential requires a considerable temporary increase of energy and protein intake that is not available for all and, when lacking, decreases the achievement of the genetic potential for height.

This is a different mechanism and timing from that which establishes the largely genetic difference between short and tall adults when all grow up in optimal circumstances. What controls this normal genetic difference – which is quite different from

the social class/secular trend mechanism – is unclear. It may be related to individual genetic differences relating to the various components of the growth hormone – IGF-1 growth axis.

Nutritional conditions are important also for the timing of puberty. This is strongly suggested by follow-up studies of adopted children relocated from poor areas to developed countries. J.P. Bourguignon and colleagues² observed that precocious puberty occurred in 8 of 32 children adopted in Belgium. They developed an experimental model to this phenomenon utilizing male rats, studying both hypothalamic maturation of the gonadotropic control, and testicular content of elongated spermatids. When compared to those of a small litter, pups from a large litter showed a reduced growth rate before weaning, and then a similar growth rate after weaning at 21 days, followed by earlier hypothalamic and testicular maturation at age 35 days. Refeeding at different times and reduction of litter size, which changes the feeding levels before weaning, showed that food-restricted pups when refeed resumed a normal growth rate and had an accelerated hypothalamic and testicular maturation advanced for their body size. This was observed only when refeeding had occurred before the age of weaning. This suggests that hypothalamic maturation of the gonadotropic control is sensitive to nutritional conditions during a limited critical period before the onset of puberty.

1. Tanner JM. *Horm Res.* 1992;38(suppl 1):106-115.

2. Bourguignon JP, Gerard A, Alvarez Gonzalez ML, et al. *Horm Res.* 1992;38(suppl 1):97-105.

Editor's comment: Though the relationship between nutrition and growth has been often documented and long debated, it is still not completely clear. Many data support the idea that optimal nutrition improves growth in infancy and early childhood, and, consequently, statural growth is greater in adults. The analysis by Tanner, based on several reported studies, leaves no doubt regarding this historical fact, evident from his own studies. He now adds the hypothesis of the associated role of hygienic conditions, and suggests the interrelation of morbidity and dietary events in the preschool age as a main factor for the secular trends in growth and also for the social differences in these trends. He presents a stimulating way to analyze new longitudinal data and better understand the population studies.

However, the time of onset of puberty and the height reached when sexual development starts are variable among individuals. This is important in medicine. The data reported by Bourguignon and colleagues stress the influence of appropriate early

nutrition, but with consequences different from those shown by population studies. Thus, nutrition may affect final height through different mechanisms. An interesting perspective is gained by presenting these 2 completely different studies in the same abstract.

Jean-Claude Job, MD

2nd Editor's comment: The Editorial Board of GGH has urged that we present a lead article on secular growth. This we will do in good time, but the recently published article by Dr. Tanner, and reviewed here by Dr. Job, is currently available and is a superb analysis of the quantitative and qualitative environmental factors affecting growth. Those interested in this topic now are urged to read Dr. Tanner's extensive and thorough presentation.

Robert M. Blizzard, MD

Factors Predictive of Sustained Growth in Children After Renal Transplantation

This paper reports growth data on renal transplant recipients collected from the North American Pediatric Renal Transplant Cooperative Study. Participating centers record data at the time of transplant and at 6-month intervals thereafter. Data include height, weight, serum creatinine, type and amount of immunosuppressants, and graft survival. Height data are reported as Z scores and catch-up growth is defined as a gain of 1 standard deviation (SD) or more. The first 300 of 1,553 patients with a functioning graft for at least 2 years were entered into this study: 64% were males, 24% had never been dialyzed, 55% had received a living-related donor kidney, and 15% had undergone retransplantation.

Baseline mean Z score was -2.41 ± 0.09 and the mean change in Z score at year 2 was 0.18 ± 0.06 ($P < 0.01$). Data were also analyzed for different age groups. Those < 1 year of age at diagnosis had the lowest initial Z score and the greatest improvement (approximately 1 SD). The 2- to 5-year-old group had a change in Z score of 0.5 SD, while those from 6 to 12 years had an increase of 0.1 ± 0.7 and those from 13 to 18 years had a decrease (-0.21 ± 0.08) (Table 1). Catch-up growth occurred in 50% of those < 1 year of age at transplantation, in 25% of the 2 to 5 year olds, in 16% of the 6 to 12 year olds, and in 6% of the 13 to 18 year olds. No differences were found in baseline Z scores for males or females, for those with or without prior dialysis, or in recipients of a living-related donor or cadaver kidney. As expected, those with a previous transplant were significantly shorter than those receiving an initial transplant, and children with aplastic or hypoplastic kidneys or with obstructive nephropathy had a greater initial height deficit. A 1.0 mg/dL increase in serum creatinine was associated with a 0.15 decrease in Z score following transplantation, but similar analysis using prednisone dose per kilogram of body weight did not demonstrate a significant relationship. The 112 patients who had a rejection episode in the first month posttransplantation had no significant Z score change at 2 years.

Editor's comment: This paper demonstrates improvement in height Z scores posttransplantation in a large group of young children whose end-stage renal disease was caused by a variety of disorders, but not in those above 5 years of age. Despite catch-up growth, height Z scores did not normalize in any group. The younger children, who were also those with the greatest initial baseline height deficit, were those who sustained the greatest height gain increments posttransplantation. This response is similar to that seen in growth hormone-deficient children in response to exogenous growth hormone. Unfortunately, this group of children is the group for whom posttransplantation mortality is the greatest. The authors note that most children older than 6 years of age at transplant did not show catch-up growth. Thus, as pointed out by the authors, the findings suggest that other treatment for growth failure is needed and that a controlled trial of recombinant growth hormone in children with end-stage renal disease posttransplantation may be warranted.

William L. Clarke, MD

Table 1
Relationship of Age at Transplantation to
Subsequent Growth

Age (y)	n	Baseline Z Score (mean \pm SEM)	Change in Z Score* (mean change \pm SEM)
0-1	22	3.04 ± 0.31	0.92 ± 0.31
2-5	64	2.10 ± 0.16	0.54 ± 0.12
6-12	137	2.34 ± 0.14	0.11 ± 0.07
13-18	77	2.21 ± 0.21	0.21 ± 0.08
Total	300	2.41 ± 0.09	0.18 ± 0.06

n = number of subjects.

*At 2 years after baseline.

Putative X-Linked Adrenoleukodystrophy Gene Shares Unexpected Homology With ABC Transporters

Adrenoleukodystrophy (ALD) is an X-linked disorder that leads to central nervous system demyelination and adrenal insufficiency. It can result in death within a few years, although the phenotype varies widely even within a family. The main biochemical abnormality found in this disorder is the accumulation of saturated very-long-chain fatty acids (VLCFA) due to impaired β -oxidation in peroxisomes.

ALD was the subject of the recent film, "Lorenzo's Oil." In this docudrama, a couple whose 6-year-old son, Lorenzo, has been stricken with the disease circumvent the medical establishment in an attempt to find a cure. The oil referred to in the title consists of erucic acid and oleic acid, which returned the raised plasma concentrations of saturated VLCFAs to normal. Unfortunately, this treatment (touted by the film as a cure for ALD) has since been shown in a 5-year controlled study to be ineffective for many ALD patients whose condition continues to deteriorate. Also of importance is that other patients who have not received the treatment remain stable for many years. Thus, it is likely that Lorenzo's condition remained stable due to chance and not to "cure" from the oil.

A gene that is thought to cause the ALD defect has been cloned recently by Patrick Aubourg's group in Paris. This gene was cloned utilizing a positional cloning approach, in which a gene is mapped to particular chromosomal region using restriction fragment length polymorphisms (RFLPs) and candidate genes in that region are then identified and used as probes for genes containing mutations in the affected families.

While it was originally thought that VLCF-CoA synthetase was the most likely candidate for the ALD gene, the gene identified by Aubourg's group bears no sequence resemblance to this or 3 other enzyme genes involved in peroxisomal β -oxidation. Instead, it is highly homologous with a peroxisomal membrane protein that is involved in peroxisome biogenesis and that belongs to a family of membrane proteins known as the adenosine triphosphate-binding cassette transporters. This family includes the multidrug-resistant gene product, the cystic fibrosis

transmembrane conductance regulator, and genes that map in the human major histocompatibility complex region. The members of this gene family are involved in transport of proteins, amino acids, inorganic ions, and peptides in both prokaryotes and eukaryotes. Thus, the sequence of this newly identified gene suggests that ALD may be caused by defective transport of VLCFA-CoA synthetase into the peroxisomal membrane, rather than by a deficiency in the VLCFA-CoA synthetase enzyme itself.

Mosser J, Douar A-M, Sarde C-O, et al. *Nature*. 1993;361:726-730.

Moser, HW. *Lancet*. 1993;341:544.

Editor's comment: It is unfortunate that a popular film, "Lorenzo's Oil," would present as fact a hypothesis that has not withstood the test of a controlled trial, and would seek to further widen the rift that exists today between the general public and the medical and scientific establishment. The fallacies of treatment and flagrant misinformation in this film are duly recorded under the title "Pernicious Treatment," by Fred S. Rosen in *Nature* 1993;361:695, and in a film review by Dr. Hugo Moser in *Lancet* 1993;341:544. Both of these outstanding scientists are appalled at the filmmaker's lack of responsibility in researching the truth and to patients with ALD, to physicians who are treating them, and to the United Leukodystrophy Foundation.

However, the discovery of a gene that may represent the causal factor in ALD is very exciting, and brings with it the hope that in the near future, families afflicted with this tragic illness will have access to an understanding of why the disease happens and new therapies that may be effective in all cases. Prenatal diagnosis should be possible, and gene therapy is certainly a possibility as clinical trials are underway for gene therapy in a number of other disorders involving the central nervous system.

Judith G. Hall, MD

Pregnancy After Age 50: Application of Oocyte Donation to Women After Natural Menopause

A recent study by Sauer et al has shown that women as old as 59 years retain the ability to bear children if hormone replacement therapy and in vitro fertilization of oocytes from younger donors are provided. The researchers were able to establish pregnancies in 9 out of 14 women, aged 50 to 59 years. Three of these women had delivered at the time of publication; 4 were progressing normally beyond the second trimester; and 2 of the 9 pregnancies had obstetric complications involving preterm labor, preeclampsia, growth retardation, and/or gestational diabetes. Of the 7 pregnant or delivered women, 4 had never previously conceived.

As might be expected, however, there did seem to be a "male factor" in the study, as the partners who donated the sperm were also over age 50. A low overall fertilization rate was noted, with most of the unfertilized eggs occurring among the 5 couples who were unable to conceive.

The authors conclude that there is little doubt that the uterus remains receptive to embryo implantation and can sustain normal pregnancy well beyond the limits of natural reproduction. They thus conclude that it is the aging of the ovaries and

oocytes, and not the uterus, that is responsible for most adverse fertility events associated with aging. Although there have been no serious complications, the authors do indicate that there are not enough data at this time to presume that the incidence of adverse results will not increase in women over 50. The psychologic consequences of giving birth after age 50 are also discussed in the paper, and the point is made that many children are raised by grandparents in various cultures.

Sauer MV, Paulson RJ, Lobo RA. *Lancet*. 1993;341:321-323.

Editor's comment: This study demonstrates that women well beyond natural menopause may still achieve implantation of transferred embryos and carry these pregnancies to term. As the authors note, the average life expectancy and quality of life in our society are increasing, but physiology has limited women in their 50s and beyond. This new technology allows women the same range of choices that men have always enjoyed: the chance to concentrate on a career without worrying as much

about the "biological clock," or the chance to have children with a second partner, should a woman be divorced or widowed.

On the negative side, however, there are costs that must be considered as well. *In vitro* fertilization technology is extremely expensive. In addition, the authors note that extensive medical and psychologic screening should be conducted with women of advanced age who are considering pregnancy; the screening

process is also very expensive. At a time when we are realizing that we must somehow curtail skyrocketing medical costs — even to the point where rationing of health care is being considered — such expensive elective procedures must be carefully evaluated and debated.

Judith G. Hall, MD

Short-Term Growth Hormone Treatment Does Not Increase Muscle Protein Synthesis in Experienced Weight Lifters

Yarasheski et al studied whether recombinant human growth hormone (GH) administration enhances muscle protein anabolism in experienced weight lifters. The fractional rate of skeletal muscle protein synthesis and the whole body rate of protein breakdown were determined using a constant intravenous infusion of C^{13} leucine in 7 young adult males who were experienced weight lifters. The studies were performed at the beginning and at the end of 14 days of subcutaneous GH administration at $40 \mu\text{g/kg/d}$, which is the dosage used often in treatment of GH-deficient patients (0.3 mg/kg/d). GH administration increased fasting serum insulin-like growth factor 1 (IGF-1) levels (Figure 1), but did not increase the fractional rate of muscle protein synthesis or reduce the rate of whole body protein breakdown from 103 ± 4 to $108 \pm 5 \text{ mol/kg/h}$. The authors state that the findings suggest that short-term GH treatment does not increase the rate of muscle protein synthesis or reduce the rate of whole body protein breakdown, metabolic alterations that would promote muscle protein anabolism in experienced weight lifters attempting to further increase muscle mass.

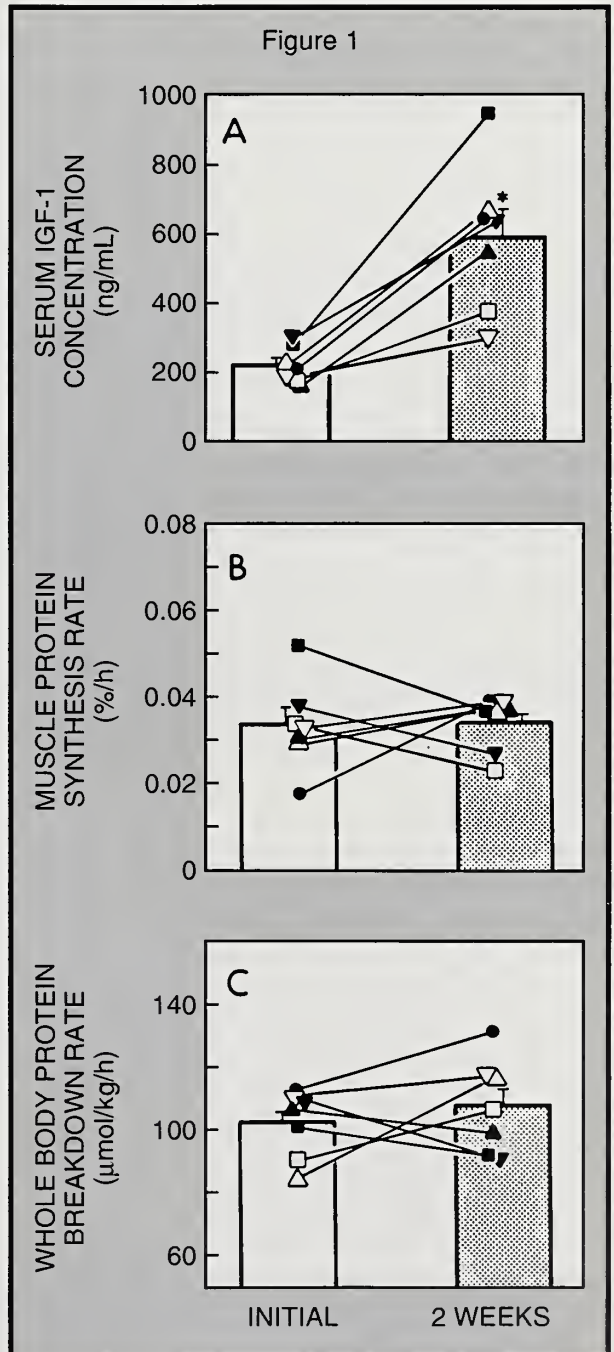
Yarasheski KE, Zachwieja JJ, Angelopoulos TJ, et al. *J Appl Physiol.* 1993;74:3073-3075.

Editor's comment: These authors previously reported that recombinant human GH at $40 \mu\text{g/kg}$ given 5 d/wk to healthy sedentary young men in conjunction with a 12-week muscle-building exercise program produced increments in muscle protein synthesis rate and muscle strength comparable to those achieved by sedentary young men doing an identical muscle-building exercise program but receiving placebo injections. However, the earlier study did not exclude the possibility that GH administration might augment muscle protein synthesis during the early phase of treatment, since muscle protein synthesis was determined only before and after 3 months of GH treatment.

The previous study (*Am J Physiol.* 1992;25:E261-E262, abstracted previously in *GGH.* 1992;8[1]14) did not consider the possibility that GH administration might enhance muscle protein synthesis in experienced weight lifters or bodybuilders who had already achieved a large muscle mass using heavy resistance exercise training or that GH administration might further increase muscle mass by supplementing with another potential anabolic stimulus. This study is important because skilled weight lifters and bodybuilders represent the most likely abusers of GH for muscle anabolism.

As is characteristic of these authors, the studies were done in an exquisite manner. The data speak for themselves. GH in such patients is not of value in increasing muscle mass. Pass the word along to the athletes who wish to spend astronomical sums of money in the hope that they will increase their competence.

Robert M. Blizzard, MD



MEETINGS CALENDAR

October 5-9, 1993 43rd Ann Mtg of ASHG, New Orleans, LA. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

October 28-31, 1993 Somatotrophic Axis & the Reproductive Process in Health & Disease, Baltimore, MD. Info: Dr B Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

November 7-11, 1993 Wkshp on the Superfamily for Receptors of GH, Prolactin, Erythropoietin & Cytokines, Haifa, Israel. Info: M Zur. Tel: 972-3-635-5038; Fax: 972-3-535-1103.

November 14-17, 1993 45th Postgrad Assembly of the Amer Endocrine Soc, San Francisco, CA. Info: C Huck. Tel: 301-571-1803; Fax: 301-571-1869.

December 9-12, 1993 GHRH, GH, IGF-1: Basic & Clin Advances, San Diego, CA. Info: Dr B Burnett. Tel: 617-982-9000; Fax: 617-982-9481.

February 6-10, 1994 3rd Intl Symp on Insulin-Like Growth Factors, Sydney, Austral. Sci Info: Dr R Baxter. Fax: 61-2-516-1273. Genl Info: E Loveridge. Tel: 61-2-956-8333; Fax: 61-2-956-5154.

March 13-15, 1994 March of Dimes Clin Genet Conf, Kissimmee, FL. Info: C Blagowidow. Tel: 914-997-4524; Fax: 914-428-8203.

March 15-17, 1994 Amer Coll of Med Genet, 1st Ann Mtg, Kissimmee, FL. Info: E Strass. Tel: 301-571-1826; Fax: 301-530-7079.

May 2-5, 1994 APA/APS/SPR Ann Mtg, Seattle, WA. Info: D Agnostelis. Tel: 708-427-1205; Fax: 708-427-1305.

June 1-4, 1994 1st Intl Mtg of the GH Research Soc, Aarhus, Denmark. Info: Drs J Christiansen/J Jorgensen. Tel: 45-86-1255-55/Ext 2084; Fax: 45-86-1378-25.

June 8-14, 1994 54th Ann Mtg of the ADA, New Orleans, LA. Info: ADA. Tel: 703-549-1500/Ext 330. Fax: 703-836-7439.

June 15-18, 1994 76th Ann Mtg of the Amer Endocrine Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1803; Fax: 301-571-1869.

June 22-25, 1994 33rd Ann Mtg of the ESPE, Maastricht, The Netherlands. Info: Prof J Van den Brande. Tel: 31-30-32-0521; Fax: 31-30-33-4825.*

July 17-24, 1994 3rd Eur Cong of Endocrinol, Amsterdam, The Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

August 20-25, 1994 7th Intl Conf on Obesity, Toronto, Can. Info: CME Office. Tel: 416-978-2719; Fax: 416-971-2200.

September 24-29, 1994 9th Intl Cong on Hormonal Steroids, Dallas, TX. Info: Dr E Simpson. Tel: 214-648-3260; Fax: 214-648-8683.

October 30-November 3, 1994 46th Postgrad Assembly of the Amer Endocrine Soc, Toronto, Canada. Info: W Johnson. Tel: 301-571-1807; Fax: 301-571-1869.

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Relevance of the Genetics of Embryologic Development

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GROWTH, Genetics, & Hormones is an appropriate title under which to present advances in developmental genetics since many developmentally important genes have been recognized through their hormone-like influence on cell growth. The connection between genes, hormones, and birth defects is well illustrated by the virilization of female fetuses with autosomal recessive 21-hydroxylase deficiency.¹ That genetic factors contribute substantially to human malformations cannot be disputed.

Some 721 single birth defects and 1,040 syndromes are included among the 3,500 mendelian disorders catalogued by McKusick,² comprising almost 50% of the total.³ The genetic contribution to birth defects is further underscored by the discovery that many malformations occurring in the absence of a positive family history (ie, sporadic malformations) actually result from spontaneously arising gene rearrangements and/or are now known to demonstrate parent-of-origin effects from inactivation of either the maternal or paternal allele of a gene through genomic imprinting.⁴ Now that most genes found to regulate development of lower animals, such as arthropods and nematodes, are being identified in humans, it becomes possible to explore their potential role in human development and to examine similarities between developmental abnormalities in these species and in humans.

DEVELOPMENTAL GENES AS GROWTH FACTORS

The roundworm *Caenorhabditis elegans* has a small genome and a transparent embryo, which allows observation of virtually all developing tissues.⁵ Among many interesting mutations identified in this species are those that delay or accelerate the timing of development (heterochrony). For example, a timing mutation called *lin-12* alters genital development. The DNA sequence of the gene involved exhibits homology with mammalian epidermal growth

Letter From the Editor

In Dr. Wilson's article, homeobox genes are referred to for one of the first times in *GGH*. These important genes control fundamental aspects of development, such as segmentation of the blast or embryo. These regulatory genes affect the activation of multiple target genes and the differentiation of certain cell types. Most genetic mutations cause damage more directly by producing defects in enzymes or receptors. One of the important aspects of understanding the action of these genes is that multiple cells may be affected, as reported from a defect in a homeobox gene known as *Pit-1*. The protein product of the *Pit-1* gene is abnormal; theoretically, it may not bind to DNA or, if it does bind, it cannot effectively activate transcription. Both the Snell and Jackson dwarf mice have abnormal *Pit-1* genes that lead to multiple hormone defects.

Continued on page 2

In This Issue

The Importance and Methods of Using Animal Models to Study

Human Disease pg 6

Abstracts pg 10

Meetings Calendar pg 16

Letter From the Editor (continued)

Defects in other homeobox genes occur, such as the *Hox 1.5* gene that produces mice fetuses without thymus and parathyroid glands and with defects of the heart and arteries to produce a DiGeorge-like syndrome. You as a reader of *GGH* and of Dr. Wilson's article may wish to read further about defective homeobox genes and the anatomic and metabolic abnormalities that result. The following references provide further insight into the importance of these organizational genes.

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factors that also provide extracellular signals during mammalian development. Similar sequences occur in the *Notch* neurodevelopmental gene of the fruit fly and in several human genes. These genes may be candidates for human birth defects thought to result from arrested development.⁶ The developmental pathways they regulate may be potential targets for therapy to prevent the occurrence of the defects.

DEVELOPMENTAL GENES AS BLUEPRINTS

The fruit fly *Drosophila melanogaster* has been used extensively to analyze the effects of genes on development. Indeed, *Drosophila* geneticists have defined 5 groups of genes that regulate early pattern formation – the genes that specify the fruit fly basic body plan.⁷ Despite obvious differences in the shapes and sizes of mammals and insects, the discovery of human counterparts to many of these fruit fly genes suggests that they are relevant to human development (Figure 1).

Pattern formation in insects begins prior to fertilization, when follicle cells surrounding the female germ cells orient oocyte cytoplasm according to “head” and “top” of the future embryo. In other words, expression of maternal genes regulates the initial stages of embryogenesis (Figure 1, column 1). Mutations in these maternal genes may cause headless embryos, like those produced by the *caudal* mutation, or deficient ventral structures exemplified by the *dorsal* mutation. After fertilization, products from several groups of embryonic genes (which have been named from their spatial or temporal expression pattern, their putative functions, or phenotypes resulting from mutations) orchestrate the next step in development, which is segmentation. First, gap genes (Figure 1, column 2) such as *kruppel* demarcate major embryonic regions. Next, pair-rule and segment polarity genes (Figure 1, columns 3 and 4) specify body segments, with appropriate anteroposterior orientation (polarity).⁷ Mutations in these genes disrupt the formation of structures normally derived from the different body segments. For instance, mutations in the gene *wingless* (now called *Wnt-1* because of its homology to the integration site for mouse mammary tumor virus) alters thoracic segments so that wings do not develop. Finally, the expression of homeotic genes gives identity (labia, antennae, limbs) to the segmental units (Figure 1, column 5). The term homeotic refers to the development of normal structures at abnormal locations. For example, normal legs form where antennae normally reside in the *antennopedia* mutations.⁸ Characterization of several homeotic genes revealed a highly conserved DNA sequence, which was named the homeobox.⁸

Figure 1
Gene Families Responsible for
Drosophila Segmentation and Their Mouse
and Human Homologues



When DNA probes designed to detect the *Drosophila* homeobox sequence were employed to search for similar DNA sequences in other species, it was discovered that organisms as diverse as frogs, mice, and humans had such genes. Indeed, 4 major clusters of homeobox-containing genes have now been found in mice and humans; they have been termed *Hox* and *HOX* genes, respectively (Figure 1, column 5). The nomenclature for these homeobox-containing genes has been confusing since they were named originally in order of discovery. Several were initially assigned to the wrong cluster and different schemes were used for different species and by different investigators. However, consensus has been reached on a naming scheme in which the clusters are called A, B, C, and D and the individual gene loci are designated numerically according to their physical location (3' to 5') within a given gene cluster, hence A1,2,3,...; C4,5,6,..., etc. This scheme assumes that each cluster will have 13 gene loci. The human *HOXA* through D clusters map respectively to chromosomes 2, 7, 12, and 17 (Figure 1). Although the functional significance of *HOX* genes in humans remains to be proven, the structure and organization of the *HOX* clusters in humans is remarkably similar to that of other species, such as the mouse and in *Drosophila*, where function has been implied from analysis of segmental expression patterns and mutations.^{7,8}

Given the homeobox as a paradigm, investigators sought and found DNA sequences in mammalian genes that corresponded to sequence motifs of other *Drosophila* genes involved in pattern formation. Such sequence similarities in different species are termed homologies, and their genes homologues. One such configuration was detected near the homeobox sequence of the *Drosophila* pair-rule

gene, *paired*; it was called the paired box or *Pax* motif. Eight mouse *Pax* genes (*Pax1* through *Pax8*) have so far been found (Figure 1).⁹ In some instances, the DNA sequences of certain oncogenes (ie, mammalian *c-rel*, *GLI*, *Wnt-1*) were found to be homologous to those of insect developmental control genes (ie, insect *dorsal*, *kruppel*, *wingless*). In short, some oncogenes are actually pattern genes in disguise. Of interest to endocrinologists is another sequence motif, the POU box, which is common to pituitary-specific transcription factors. These factors can affect synthesis of pituitary proteins (ie, prolactin, growth hormone) in nonendocrine cells such as HeLa cells.¹⁰ Just as the paired box and homeobox motifs occur together in the fruit fly gene *paired*, a POU box and homeobox occur together in pituitary transcription factors.

As shown in Figure 1, there are now mammalian homologues for each group within the hierarchy of genes that specify the *Drosophila* body plan. Presumably, these function to organize the basic body plan in mammals much like they do in insects, but the precise roles of the gene products are not known. Thus, at the current time, human development is like a repertory theater: the actors are known but not their roles in a given performance.

FUNCTIONAL TESTS FOR DEVELOPMENTAL GENES

The mouse is an ideal model with which to study human development since its development closely resembles that of humans yet occurs over a time span short enough for analysis. Recent advances in molecular genetics that facilitate manipulation of the mouse genome have provided a means to explore the functions of putative mammalian developmental genes. In short, a gene of interest is altered, introduced genetically into (transgenic) mice, and the effects on development analyzed in progeny that express the mutation. In experiments using transgenic techniques, the DNA of an exogenous gene, the transgene, is introduced into a mouse egg. In this type of work genes can be inactivated, or knocked out, to examine what happens when the normal function of the gene is lost (loss-of-function mutations). Alternatively, genes can be over-expressed by joining them to regions of DNA that promote expression of the gene (promoters), causing them to be expressed at a high level. This produces gain-of-function effects. These technologies have been used to generate a number of mutant mice that have provided insight into mammalian development, with implications for human birth defects. For example, overexpression of the *Hox 1.1* gene produced mice with small jaws reminiscent of the Pierre Robin syndrome in man.⁹

Table 1

Gene(s)	Function	Developmental Process Affected	Human Malformation
<i>Oct-3</i>	Transcription factor	Zygote cleavage	
<i>Wnt-1</i>	Signal transduction	Axis formation	
<i>Xhox</i>	Transcription factor	Axis formation	
<i>FGFs</i>	Signal transduction	Gastrulation	
<i>Hox(s)</i>	Transcription factor	Segment specification	
<i>Pax(s)</i>	Transcription factor	Segmentation	
<i>NF-1</i>	Signal transduction	Cell growth	Neurofibromatosis
<i>WT-1</i>	Transcription factor	Differentiation	Wilms' tumor
<i>SRY</i>	Transcription factor	Differentiation	Gonadal dysgenesis
<i>GLI</i>	Transcription factor	Differentiation	Grieg syndrome
<i>KALIG-1</i>	Cell adhesion	Cell migration	Kallmann syndrome
<i>c-kit</i>	Signal transduction	Cell migration	Piebald trait
<i>COL1A1</i>	Mechanical integrity	Tissue growth	Osteogenesis imperfecta
<i>Fibrillin</i>	Mechanical integrity	Tissue growth	Marfan syndrome

Changes in the cervical vertebrae were suggestive of anterior-to-posterior segment transformations caused by homeotic mutations in the fruit fly. Deficient expression of the *Hox 1.5* gene produced mice with absent thymus, missing parathyroids, and cardiac anomalies. The phenotype was similar to that of the human DiGeorge syndrome.

Mutations have also been characterized in several naturally occurring mouse mutants. The phenotype of the mouse *undulated* mutant with its severe vertebral anomalies has been explained by a mutation in the *PAX-1* gene (Figure 1). The coat color and hematologic defects in the *W* mutation have been mapped to the *c-kit* oncogene (Table 1).⁹

APPLICATION TO HUMAN BIRTH DEFECTS

Table 1 lists selected genes or gene families that regulate or otherwise participate in developmental processes. Mutations in some genes have been found in human genetic disorders. It must be emphasized that the number of developmentally important gene families and the number of genes within such families is growing rapidly, and the assignment of human malformations to mutations in these genes is only beginning to emerge. The mutations identified to date highlight 3 major avenues for future investigation. The first is correlating normal function, or loss of function, of a gene product with a recognized clinical phenotype. For example, the fragile bones of osteogenesis imperfecta correlate well with loss of mechanical strength normally provided by type 1 collagen in bones. Similarly, the pie-bald trait correlates with loss of influence of the *c-kit* oncogene protein on melanoblast migration. Another example is the loss of the regulatory function of the G protein-related product of the *NF-1* gene in neurofibromatosis.

The second avenue is the use of DNA sequence and/or protein structure to identify human genes exhibiting homology with developmental control genes defined in lower species. The Grieg syndrome, with craniosynostosis and digital anomalies, has been traced to the *GLI* oncogene family that is homologous to the *kruppel* gene of the fruit fly (Figure 1).¹¹

The third approach is the use of genetic linkage or chromosomal rearrangements to map putative developmental genes. Chromosomal deletions were important in defining the *WT-1* gene involved in kidney embryogenesis, the *SRY* gene responsible for male sex determination, and the *KALIG-1* gene that influences the migration of neural cells that contribute to olfaction and gonadotropin secretion. Mutations in these genes occur in Wilms' tumor, gonadal dysgenesis, and the X-linked Kallmann syndrome, respectively.¹²

For most developmental disorders, a combination of the gene function-phenotype correlation (candidate gene), gene structure (DNA sequence homology), and gene localization (reverse genetic, or position cloning) approaches will be used to identify genes of potential developmental importance in humans, with mouse genetic manipulation (transgenic and gene-targeted mice) as a key arbiter of their functional significance.

A closing example will illustrate how these developmental approaches offer new perspectives on complex disorders. Situs or laterality defects comprise a large spectrum, including multiple or absent spleen(s), pulmonary isomerism, cardiac defects (ie, transposition, anomalous pulmonary venous return), midline liver, and intestinal malrotation. Pure situs inversus viscerum or syndromes of mixed situs (heterotaxy-Kartagener and Ivemark syndromes) can exhibit familial patterns consistent with

autosomal recessive inheritance. One affected fetus has been described with a translocation breakpoint at chromosome region 12q13.¹³ The similar mouse *inversus* (*iv*) mutation has been explained as a change from directed to random situs and linked to the immunoglobulin heavy chain locus on mouse chromosome 12 (human 14q32).¹⁴ The theory of Brown and Wolpert,¹⁵ derived from a variety of developmental systems, provides an approach to this spectrum of human malformation.

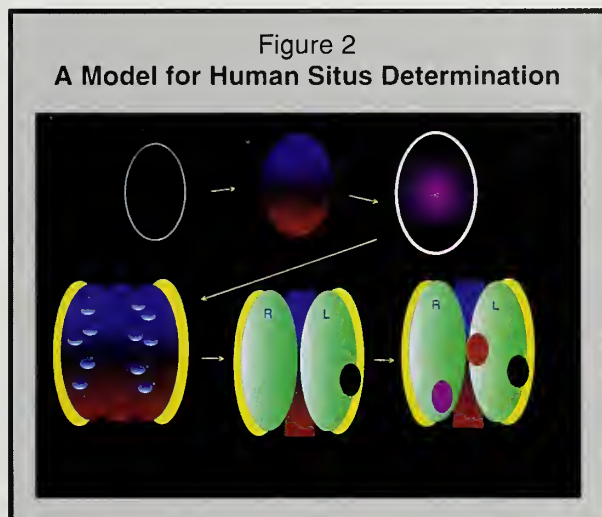
As diagrammed in Figure 2, the first steps towards right-left differences involve specification of the anteroposterior (A-P) and dorsoventral (D-V) axes. Mutations that reverse the A-P or D-V axes would produce pure situs inversus without functional consequences. The next step involves development of a midline axis and bilateral symmetry, probably before the primitive streak stage. Monozygotic or conjoint twinning are examples of midline axis disruption, and both are associated with situs defects.

Once bilateral symmetry is established, a handed (asymmetric) molecule¹⁵ is postulated that transports a morphogen or contracts in one direction (rightwards in Figure 2). In the case of morphogen transport, note that high concentrations of morphogen will occur at the midline on the right and at the periphery on the left (Figure 2). If the combination of high morphogen-peripheral molecule signals formation of a spleen, then this is a mechanism for splenic situs. Mutations inactivating the asymmetric molecule or its morphogen gradient would cause random situs determination as observed in the human and mouse heterotaxias. Failure of localized embryonic regions to respond to these signals would lead to isolated laterality defects such as malrotation or dextrocardia. This stepwise transformation of equivalent body halves is reminiscent of segment transformation in *Drosophila* and relevant to the presence of a human homeotic cluster at chromosome band 12q13. No homeobox sequences have been detected on mouse chromosome 12 or human 14q32, but characterization of the mouse *iv* gene is underway using the approaches outlined in this article.¹⁴

SUMMARY

The explosion of new knowledge about the developmental genetics of simpler organisms has revolutionized the approach to studying human malformations. The discoveries have unified biology by implicating the same or similar genes in controlling the formation of diverse body plans, by revealing new relationships such as that between tumor growth and embryonic differentiation, and by dramatizing the partnership between clinical medicine and basic science. Although certain human malformations will be irreversibly determined at the

earliest stages of zygote cleavage, they will be detectable by preconceptional, preimplantation, and/or prenatal screening. Other birth defects may be responsive to maternofetal therapy with novel hormones, growth factors, and other treatment strategies. Advancing this multispecialty enterprise of gestational endocrinology should be a highlight of 21st century medicine.



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Letter to the Editor

We read the appraisal by Professor MacGillivray¹ of the clinical significance of urinary growth hormone measurements with great interest and agree with many of her observations and reservations. The fraction of plasma GH excreted in the urine is very small, renal factors may account for 50% of the variability of excretion,² and there is a wide range of urinary GH values in normal children. It is surprising that the tiny fraction of GH excreted in the urine is so well correlated to plasma levels over the collection period.

However, what Professor MacGillivray considers to be of clinical significance could be debated. She suggests that urinary GH measurements fail to distinguish between a group of short normal children who have suboptimal spontaneous production of GH from short normal children who have normal GH production on serial blood sampling over a 12- to 24-hour period. The only reason why this distinction might be of relevance is presumably to select the former children for GH treatment, but as far as we are aware, there is no evidence that such children derive greater benefit from additional GH than any other group of short normal children. Therefore, the distinction is not of proven clinical benefit or usefulness.

On the other hand, it is very important to distinguish classical GH deficient children from short

normal children, however subdivided, because GH treatment in the latter group must still be regarded as experimental and has not yet been proven to be of long term benefit. We have shown that urinary GH measurements, using sound age- and sex-related reference ranges for comparison,² are useful in defining what Professor MacGillivray calls "severe" GH deficiency and indeed have a better predictive value (89% vs 45%) than stimulation tests.³ We have regarded the near total overlap of values in short normal children with values for the whole population to be of clinical value rather than the reverse.

However, the case for the clinical use of urinary GH measurements should not be overstated. The jury is still out, but there are grounds for mild optimism. The case against the use of a hypoglycemic stimulus of GH release has been proven.⁴

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The Importance and Methods of Using Animal Models to Study Human Disease

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Increasingly, animal models are being used to provide insights into the pathogenesis of human endocrinologic disorders as well as bone dysplasias and malformations, and in the laborious process of gene mapping and isolation. The most important mammalian model is the laboratory mouse, although other species also are coming under study. This brief review reports some of the major successes in using mouse models in the study of human disease.

CANDIDATE GENES AND CHROMOSOME HOMOLOGY

Although there are 23 pairs of human chromosomes and only 20 pairs of mouse chromosomes, and the

overall appearance of the chromosomes is different, segments of chromosome have been identified in mice and humans that appear to have been conserved because they contain 2 or more identical marker loci in both species. Such *syntenic segments* can be used to provide clues to gene mapping. If a human disorder is homologous to a mouse mutant, and the mouse mutant has been mapped and attributed to a syntenic segment, then the gene location on the mouse chromosome will point to a putative location for the gene on the human chromosome. For example, several mouse mutants with pigmentary and inner ear abnormalities show homology to the Waardenburg syndromes.¹ Type 1 Waardenburg syndrome was localized to 2q37 in a syntenic region that in the mouse contains the *spotch* (*Sp*) mutant.² Because the *Pax3* gene in the mouse mapped close to the *Sp*, it was investigated and mutations were found in 2 different alleles of *spotch*.³

Tassabehji et al⁴ and Baldwin et al⁵ then demonstrated mutations in the HuP2 gene (which is the homologue of the mouse *Pax3* gene) in human type 1 Waardenburg syndrome.

The study of mouse paired box (*Pax*) genes and their human equivalents has been extremely fruitful.⁶ Like homeobox genes, *Pax* genes were originally discovered in *Drosophila*. They code for proteins containing a DNA-binding domain, and as such are transcription factors that turn on other genes to regulate embryologic development. There are at least 6 *Pax* genes in mice and humans; in addition to *Pax3* mutations, 2 other *Pax* genes have been found to be mutated and associated with congenital anomalies. Mice homozygous for mutations of the *Pax1* gene show the undulated (*un*) phenotype with a shortened kinky tail, kyphosis due to vertebral defects, and absence of acromion scapulae. *Pax6* mutated in small eye (*Sey*) in the mouse and in dominant aniridia, mapping to 11p13 in the human.⁶

Another important example of the identification of development genes through comparative gene mapping and candidate genes is that afforded by the study of retinitis pigmentosa. This disorder is heterogeneous, with several autosomal dominant, recessive, and X-linked forms recognized. The mouse mutant retinal degeneration-slow (*rds*) was found to be due to a mutation at the locus for a membrane protein, peripherin. Mutations at this locus were also found in some human families with autosomal dominant retinitis pigmentosa.⁷

GENETIC MARKERS AND CONTROLLED MATING

Mapping genetic disorders depends on being able to study a large number of genetic markers that segregate in matings producing affected animals. This means not only that the markers themselves must be available but also that the study animals should be heterozygous for these markers.

A detailed map of the mouse genome is rapidly being constructed using variable number tandem repeat (VNTR) probes,⁸ microsatellite repeats,⁹ and polymorphism around stably inserted murine leukemia proviruses.¹⁰

As mouse strains are inbred, most animals will be homozygous at marker loci. This disadvantage has been largely overcome by 2 techniques: interspecific crosses and recombinant inbred strains. The laboratory mouse *Mus musculus* will mate with the species *Mus spretus*. As the 2 species have diverged over several million years, there are different alleles at most marker loci; the offspring will be heterozygous at most marker loci, allowing the mapping of disease loci in the laboratory mouse parent. Recombinant inbred strains are derived by inbreeding the offspring of crosses between 2 laboratory

strains of mice. Different strains will have different alleles at many marker loci.

When 1 of the parental strains has a specific disease susceptibility for a polygenic disorder, recombinant inbred strains can be used in order to identify the loci responsible for the susceptibility to particular chromosome segments.

COMMON DISORDERS

Common diseases with a polygenic component, such as diabetes mellitus, epilepsy, and hypertension, have long been considered a geneticist's nightmare because of the difficulty in analyzing the interaction of several genetic loci with environmental factors. The ability to set up specific matings in mice, together with the increasing specificity of the mouse genetic map, is allowing investigators to tackle some of these problems.

Examples of these studies include work on the non-obese diabetic (NOD) mouse, which is an animal model of type 1 diabetes,¹¹ and work on the mutant diabetes (*db*) mouse, which is a possible homologue of type 2 diabetes.^{12,13} The epileptic (*El*) mouse has been studied as a model of human temporal lobe epilepsy. Two susceptibility genes have been identified, one each on chromosomes 2 and 9.¹⁰

Genetic susceptibility to infection can also be studied more easily in mice. Alleles at a single locus on mouse chromosome 1 (*Lsh*) appear to confer resistance to leishmaniasis, salmonellae, and tuberculosis.¹⁴ It has been suggested that this locus is involved in macrophage activation. There is some evidence of a human homologue to this gene.¹⁵

Rat strains that are naturally hypertensive and prone to stroke have been used to study the genetics of these traits by using linkage analysis. Susceptibility genes have been identified both on the rat X chromosome and on chromosome 10 in a region homologous to human chromosome 17q. The human angiotensin 1-converting enzyme also maps to 17q, and the rat homologue of this gene maps close to the hypertension gene on rat chromosome 10.¹⁶

CONSTRUCTING ANIMAL MODELS

The cloning of specific genes has made feasible the creation of animal models of genetic disorders. The method used is gene targeting in mouse embryonic stem cells, ie, inserting DNA into the cells (transfecting) with constructed plasmids containing part, but not all, of the gene in question, together with selectable markers. This leads to mutation of the gene by homologous recombination in a small proportion of cells. Selected stem cells in which targeting has been successful then can be introduced into a mouse embryo. Some recipient adult mice will be mosaic

for the mutated gene; if they carry mutations in the germ cells, a proportion of their offspring will carry the mutation in every cell. A mouse model of Gaucher disease has been created in this way,¹⁷ and a mouse model of cystic fibrosis has recently been reported.

The same technique is being used to study the effects of genes responsible for embryologic development. For example, LeMouellic et al¹⁸ introduced a null mutation into the mouse *Hox 3.1* gene. They demonstrated in homozygous mice that there was an anterior shift of trunk segments, an example of which was the appearance of an extra pair of ribs attached to the first lumbar vertebra. This approach also was used to prove that the testis-determining factor (Tdf) gene was the only segment of DNA needed to change a female XX mouse into a male.¹⁹

DIFFERENCES IN MANIFESTATION OF GENE-SPECIFIC MUTATIONS

Direct molecular homology between mouse and human mutations does not guarantee phenotypic similarity. For example, the *mdx* mouse has no dystrophin in skeletal muscles and the resultant disease is directly homologous with Duchenne muscular dystrophy,²⁰ yet little detectable muscle weakness or progressive morphologic abnormality (apart from the diaphragm) is observed, although these are major components of Duchenne muscular dystrophy.

Another example is that the hypoxanthine-guanine phosphoribosyl-transferase (*Hprt*)-deficient mouse shows none of the clinical effects seen in humans with Lesch-Nyhan syndrome, which has the same enzyme defect. This presumably is due to alternative metabolic pathways in the mouse.²¹ Another variant in the difference of expression by the same gene-specific mutation occurs in respect to mode of inheritance. Various hemolytic anemias due to red cell defects are directly homologous in mice and humans (see Table 1).²² However, the mode of inheritance of spherocytosis types 1 and 2 in the mouse is autosomal recessive, whereas in humans it is autosomal dominant.

It has been suggested that some pathologic processes are dependent on absolute time rather than relative (biologic) time.²³ If this is true, it may be impossible to produce a convincing mouse model of some human genetic disorders with late onset, such as Huntington disease.

CONCLUSIONS

Many potential animal models of human disease exist, but these have yet to be associated with their human counterparts because they have not been exhaustively studied.^{24,25} Sometimes a mouse mutant can point to the etiology of a human abnormality

that has not previously been contemplated. For example, it has been suggested that some infants have a constellation of abnormalities similar to those caused by the mouse mutant *Disorganization*.²⁶ An infant was reported with right-sided tibial hypoplasia with a high degree of polydactyly of the toes on the same side. An ectopic digit was situated on the lower abdomen. Mice heterozygous for the *Disorganization* gene have similar defects, and may even have complete limb duplication, demonstrating that apparently teratomatous lesions can be caused by a single gene.

Potential homologues are first recognized at the phenotypic level; further confirmation of true homology can be obtained by comparative gene mapping and, ultimately, by demonstrating homology at the level of the gene sequence in both species. Examples of mouse models identified at 1 of 3 levels – phenotype, genotype, and mapping – are presented in Table 1.

The ability to construct specific matings in the mouse also allows for the genetic analysis of multifactorial “common” disorders, a subject well reviewed by Todd.²⁷

In summary, both gene mapping and the new techniques recently developed to study molecular biology have placed us on the threshold of dissecting much of the pathophysiology that we as physicians observe. The same mapping and techniques utilized in animal models permit correlations and conclusions that otherwise would not be possible. These cannot be ignored by physicians dealing with congenital anomalies or errors in metabolism.

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Table 1
Some Human Disorders and Proposed Mouse Homologues

		<u>Basis of Homology</u>
<u>Hematological Disorders</u>		
Chediak-Higashi syndrome	<i>bg</i> - beige	Phenotype
Hermansky-Pudlak syndrome	<i>coa</i> - cocoa, <i>pe</i> - pearl, <i>sdv</i> - sandy	Phenotype
Wiskott-Aldrich syndrome	<i>sf</i> - scurfy	Mapping
Spherocytosis (beta-spectrin def)	<i>ja</i> - jaundiced	Genotype
Spherocytosis (ankyrin def)	<i>nb</i> - normoblastic anaemia	Genotype
Spherocytosis (alpha-spectrin def)	<i>sph^{ha}</i> - spherocytosis	Genotype
<u>Endocrine Disorders</u>		
Familial idiopathic gonadotropin deficiency	<i>hpg</i> - hypogonadal	Phenotype
Type 2 diabetes	<i>db</i> - diabetes	Phenotype
Type 1 diabetes	<i>nod</i> - nonobese diabetic	Mapping
Pituitary dwarfism	<i>mn</i> - miniature	Mapping
Testicular feminization	<i>Tfm</i> - testicular feminization	Genotype
<u>Metabolic Disorders</u>		
X-linked hypophosphatemic rickets	<i>Hyp</i> - hypophosphatemia, <i>Gy</i> - gyro	Mapping
Krabbe disease	<i>twi</i> - twitcher	Genotype
Menkes syndrome	<i>Mo</i> - mottled	Mapping
Lesch-Nyhan syndrome	<i>Hprt</i> - hypoxanthine guanine phosphoribosyltransferase deficiency	Genotype
MPS type VII	<i>gus^{mps}</i>	Genotype
Ornithine transcarbamylase deficiency	<i>spf</i> - sparse fur	Genotype
Carbonic anhydrase deficiency	<i>Car-2ⁿ</i> - carbonic anhydrase II null allele	Genotype
Pelizaeus-Merzbacher disease	<i>jp</i> - jimpy	Genotype
Phenylketonuria	<i>Pah</i> - phenylalanine hydroxylase	Genotype
<u>Skeletal Abnormalities</u>		
Cleidocranial dysostosis	<i>ccd</i> - cleidocranial dysplasia	Phenotype
Osteopetrosis	<i>gl</i> - grey lethal	Phenotype
Osteogenesis imperfecta	<i>fro</i> - fragilitas ossium	Phenotype
Conradi disease	<i>Bpa</i> - bare patches	Mapping
<u>Limb Abnormalities</u>		
Fraser syndrome	<i>my</i> - blebs, <i>bl</i> - blebbed	Phenotype
Split hand and foot	<i>Dac</i> - dactylaplasia	Phenotype
Weyers oligodactyly	<i>ol</i> - oligodactyly	Phenotype
Greig syndrome	<i>Xt</i> - extra toes	Mapping
Cenani-Lenz syndrome	<i>ld</i> - limb deformity	Phenotype
<u>Skin/Pigmentary Abnormalities</u>		
X-linked ectodermal dysplasia	<i>Ta</i> - tabby	Mapping
Incontinentia pigmenti	<i>Str</i> - striated	Mapping
Goltz syndrome	<i>Td</i> - tattered	Mapping
Piebaldism	<i>W</i> - Dominant white spotting	Genotype
Waardenburg syndrome type 1	<i>Sp</i> - splotch	Genotype
Albinism - tyrosinase negative	<i>c</i> - albino	Genotype
<u>Eye Abnormalities</u>		
Aniridia	<i>Sey</i> - small eye	Genotype
Retinitis pigmentosa (AD) (peripherin abnormality)	<i>rds</i> - retinal degeneration-slow	Genotype
<u>Miscellaneous</u>		
Spondylocostal dysostosis	<i>Mv</i> - malformed vertebrae, <i>Rf</i> - rib fusion, <i>rv</i> - rib vertebrae, <i>rh</i> - rachiterata, <i>pu</i> - pudgy	Phenotype
Situs inversus	<i>iv</i> - situs inversus viscerum	Phenotype
Duchenne muscular dystrophy	<i>mdx</i> - X-linked muscular dystrophy	Genotype

Are Children Born Small for Gestational Age at Increased Risk of Short Stature?

In Israel all children are evaluated at 17 years of age for military service, and thus the authors were able to determine adult or near-adult heights of 30 boys and 34 girls born from 1970 to 1971 at 39 weeks gestation who were small for gestational age (SGA = birth weight below the 3rd percentile for the study population) and who had no identifiable cause for being SGA. The mean height of the males at 17 years of age was

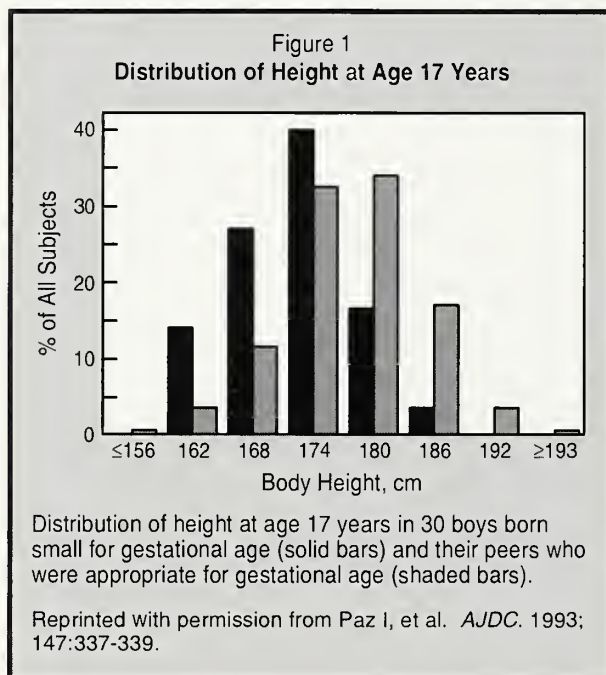
169.4 cm and that of the females was 160.3 cm. These values were 5.8 cm and 3.4 cm (respectively) less than those of control subjects of appropriate size for gestational age (AGA). The distribution of heights was shifted to the left in comparison to that of the AGA population in both sexes (Figure 1). Mean weights were 2.1 kg and 1.9 kg less than those of AGA boys and girls, respectively, but body mass indices were similar in SGA and AGA subjects. The investigators also calculated the odds ratio that SGA individuals would be less than the 10th percentile for height as an adult (4.13 for males, 3.32 for females).

Paz I, et al. *AJDC*. 1993;147:337-339.

Editor's comment: These data indicate that otherwise normal SGA infants are significantly smaller as adults than are AGA infants. Although data on bone age and stage of sexual maturation are not provided, the authors cite other data indicating that neither bone age nor age of pubertal onset is delayed in SGA subjects. Chaussain et al¹ reported that the mean adult height of 21 French SGA boys born at term was 162.8 cm and that of 23 SGA girls 147.6 cm; this is 7.2 cm and 9.9 cm, respectively, less than adult heights predicted in earlier childhood. Interestingly, the adult heights of the French SGA subjects were substantially lower than those of the SGA Israelis. Whether any therapeutic intervention would be useful or should even be considered in regard to the growth of these subjects is a matter of current investigation.

Allen W. Root, MD

1. Chaussain JL, et al. Actual versus predicted final adult height in patients with intrauterine growth retardation (IUGR). *Pediatr Res*. 1993;33:S38. Abstract 210.



Pseudotumor Cerebri and hGH Administration: A Report by the National Cooperative Growth Hormone Safety Subcommittee

Pseudotumor cerebri (PC), or idiopathic intracranial hypertension, occurs with a frequency of 1:100,000 per year in the general population. The incidence is high in obese women, but reviews of the literature report a sizable proportion of patients ages 5 to 15 years, although infants also may be affected. Permanent visual loss, which is due to damage of the optic disc as a result of papilledema is the major complication. Treatment is aimed at prevention of visual deficits and prolonged symptoms by reducing intracranial pressure. The prognosis is excellent with proper management.

Several diagnostic criteria for PC have been defined, including elevated cerebrospinal fluid (CSF) pressure without abnormal CSF composition. Normal brain appearance with normal or small ventricles is found with imaging studies. Headache, visual disturbances, and papilledema are the most common clinical findings in adults and adolescents. Infants and young children may present with irritability, apathy, or somnolence, rather than headache. Dizziness and ataxia also may occur. Older children and adolescents typically complain of headache, sometimes accompanied by nausea and vomiting. Preadolescents appear more likely than adults or adolescents to manifest symptoms other than

headache and papilledema, including lateral rectus paresis, vertical strabismus, facial paresis, and back and neck pain.

Numerous conditions and risk factors have been linked to the development of PC, including obesity or significant weight gain, steroid withdrawal, and Addison disease. The link to obesity and weight gain may be related to extra ovarian estrone production

Table 1
Growth Hormone Treatment History in Patients
With Pseudotumor Cerebri
(n=24)

Treatment Status	n=
GH therapy discontinued, symptoms improved/resolved	12
GH therapy discontinued, patients rechallenged, symptoms reappeared	4
GH therapy discontinued	7
Disposition unknown	1

in adipocytes, which is believed to stimulate CSF formation. In children, obesity does not appear to be an important factor. Conditions that may be associated with PC include hypothyroidism and iron deficiency anemia. These associations are not well established and may represent chance occurrences. Drugs also have been implicated, including corticosteroids, tetracycline, minocycline, nalidixic acid, trimethoprim/sulfamethoxazole, indomethacin, isotretinoin, danazol, and oral contraceptives.

Between 1985 and the present, Genentech, Inc. learned of 24 instances (12 female, 12 male) of "diagnosed" or "suspected" PC in patients receiving growth hormone (see Table 1). Twenty cases involved patients receiving Protropin® (somatrem for injection) therapy, 3 cases involved Humatrope® (somatropin, rDNA origin, for injection), and the type of GH was not specified in the 1 remaining case.

In 7 of the 12 patients in which GH therapy was continued, symptoms of PC resolved spontaneously over a period of months. Three patients continued to receive GH and symptoms had not resolved at the most recent examination. In 2 other cases, it was not learned whether GH had been withdrawn and restarted.

Sixteen of the 24 total PC patients were reported through the Protropin® National Cooperative Growth Study. Table 2 provides diagnoses, age, and treatment duration information. It is important to note that most of these 16 patients had 1 or more of the previously mentioned conditions or risk factors associated with PC.

The management of these patients varied but was consistent with guidelines in the literature. The most common modes of therapy are directed at relieving symptoms associated with elevated CSF pressure and at protecting vision. Therapies include acetazolamide, which reduces CSF formation; corticosteroids; periodic lumbar punctures to alleviate intracranial pressure; and, occasionally, lumboperitoneal shunting. In none of the cases reported to Genentech was surgical intervention performed, ie, subtemporal decompression or optic nerve sheath fenestration. Permanent visual loss did not occur in any of the patients.

In summary, PC has been reported rarely in patients treated with GH. Some of the patients had concurrent conditions known to be associated with PC. Therefore, the role of GH therapy is

Table 2
Reports of Pseudotumor Cerebri*
in GH-Treated Patients
(n=16)

Sex: 10 Females, 6 Males

Age: Range = 7 to 18 years; Mean = 12.5 years

Duration of Treatment: Range = 0.1 to 62 months;
Mean = 9.0 months

Diagnosis	n=
Idiopathic GH Deficiency	8
Short Stature due to Chronic Renal Insufficiency	5
Prader-Willi Syndrome	1
Turner Syndrome	2

* Data derived from the Protropin® National Cooperative Growth Study.

not known, although a possible association has not been excluded. Traditional management has been successfully employed in treating this condition; in some cases, GH therapy was discontinued.

Editor's comment: When this report crossed my desk, it seemed important enough to be abstracted. Therefore, I elected to share this information with those of you who did not read the original report. In my experience with more than 1,000 patients on hGH, I have seen PC occur only once. It regressed quickly with no change in GH dose. For those who wish to read further, a comprehensive paper from the FDA [Malozowski S, Tanner LA, Wysowski D, Flemming GA. Growth hormone, insulin-like growth factor 1, and benign intracranial hypertension. *New Engl J Med.* 1993; 329:665-666] appeared in August. This paper includes all the data available to Genentech as well as data on 3 cases of pseudotumor cerebri that occurred in subjects treated with IGF-1.

Robert M. Blizzard, MD

Gene Associated With Amyotrophic Lateral Sclerosis (Lou Gehrig's Disease) Codes for Superoxide Dismutase

Amyotrophic lateral sclerosis (ALS) is a late onset, ultimately fatal disease characterized by the degeneration of motor neurons, causing a progressive paralysis that can proceed for many years before causing death. In addition to Lou Gehrig, whose illness gave the disease its popular name, ALS is also well-known for its attack on the brilliant astrophysicist Stephen Hawking, author of *A Brief History of Time*. Most cases of the disorder are sporadic, but approximately 10% are familial.

Rosen et al have identified the SOD1 gene on human chromosome 21 that codes for cytosolic superoxide dismutase (SOD). This gene is the most likely candidate for the ALS defect. The SOD enzyme catalyzes the conversion of superoxide (O_2^-), a toxic-free radical, into hydrogen peroxide (H_2O_2), which can then be converted to water by glutathione peroxidase or catalase.

Superoxide is a highly reactive molecule involved in numerous physiologic and pathologic processes. It also is highly destructive. For example, free radicals have been implicated in

DNA breakage leading to cancer and aging, and in damage to other cellular structures. Superoxide is released in abundance during the respiratory burst of activated phagocytic leukocytes and plays a role in inflammation. Many bioactive molecules, such as nitric oxide, also react with superoxide, affecting their levels in various tissues. Reperfusion, which injures numerous tissues, also involves superoxide.

How might a defect in SOD1 contribute to the very specific pathology found in ALS? Free radicals have been proposed to cause neuronal injury in several neurological disorders, including Parkinson's disease and ischemic brain injury. Thus, one possibility is that SOD1 activity is reduced in ALS patients, leading to an accumulation of the toxic superoxide radical. Alternatively, the activity of SOD1 might be increased, leading to excessive levels of hydrogen peroxide and, subsequently, of the highly toxic hydroxyl radical. Overexpression of SOD1 in transgenic mice was found to lead to an apparently specific

defect in the motor neurons of the tongue and hindlimbs. This indicated that the SOD1 gene can selectively affect motor neurons.

In a companion piece in the same issue of *Nature*, neurologist James McNamara and biochemist Irwin Fridovich discuss another possibility for the involvement of free radicals in the neuronal injury in ALS. In acute neuronal injury due to ischemia or hypoglycemia, abnormally high concentrations of glutamate accumulate in the extracellular space and excessive activation of neuronal glutamate receptors ensues, thereby literally exciting these glutamate-receptive neurons to death. Epidemiologic evidence hinted at a link between ALS and glutamate when ingestion of excess amounts of a glutamate analogue found in certain nuts was implicated in a form of ALS and Parkinson dementia in Guam. More direct evidence of this excitotoxic mechanism for chronic injury of motor neurons was provided by studies with spinal cord explants, which showed that incubation of these cultures with a glutamate-uptake blocker, causing excess glutamate to accumulate at the extracellular receptors, selectively kills motor neurons. Apparently the glutamate binding by specific receptors requires an influx of calcium, which in turn causes generation of high levels of superoxide. The death of these neurons in culture can be prevented by the addition of SOD1. Thus, a subtle increase of superoxide free radical over the course of a lifetime, caused by decreased SOD1 activity, might lead to the slow death of motor neurons.

Rosen DR, et al. *Nature*. 1993;362:59-62.

McNamara J, Fridovich I. *Nature*. 1993;362:20-21.

Editor's comment: *The identification of the genetic defect responsible for this devastating illness sheds light on possible mechanisms for its pathogenesis, as well as potential approaches to therapy for ALS, and possibly even for other similar disorders such as Parkinson's disease. For example, antagonists of the glutamate receptors, which are inhibitors of the enzymes that cause production of superoxide free radical, or treatment with superoxide dismutase itself might be effective as*

therapies. If, on the other hand, ALS is caused by elevated levels of SOD1, therapies which block its production or activity may be useful. In addition, prenatal diagnosis for this disorder should now become possible, as about 10% of ALS cases are familial with autosomal dominant inheritance. Given the late onset of ALS, carrier testing may also be useful, since a possible carrier may wish to know his or her carrier status before conceiving children.

Judith G. Hall, MD

In Future Issues

Overgrowth Syndromes and Disorders: Definition and Classification

by David Weaver, MD

The Overgrowth Syndromes: An Update

by Kenneth L. Jones, MD

Insulin-like Growth Factor 2 and Growth

by Yves Le Bouc, MD

Neuroendocrinology of Growth Hormone Secretion

by Jesus Argente, MD, PhD

Serum Polypeptide Hormone Binding Proteins

Part 1: Growth Hormone Binding Protein Part 2: Insulin-like Growth Factor Binding Proteins

by Allen W. Root, MD

Treatment of Craniopharyngioma: End Results

by Edward Laws, MD

Trial of Insulin-like Growth Factor 1 Therapy for Patients With Extreme Insulin Resistance Syndromes

The potential glucose lowering effect of insulin-like growth factor 1 (IGF-1) in individuals with a variety of insulin resistance syndromes was studied. Eleven patients demonstrated extreme insulin resistance syndromes, including 6 with type A insulin resistance syndrome; 2 with congenital generalized lipodystrophy; 2 with leprechaunism; and 1 with an unidentified syndrome that included facial abnormalities, a mild degree of subcutaneous fat atrophy with relatively well-developed musculature, and acanthosis nigricans.

The study had 2 phases: an acute treatment phase and a long-term treatment phase. All subjects, except a 7-month-old baby with leprechaunism, were studied for the acute effects. All were admitted to the hospital and treated with a standard diet; none received any medication other than IGF-1 (recombinant human IGF-1 [FK780], Fujisawa Pharmaceutical, Osaka, Japan). After an overnight fast, patients were given a subcutaneous bolus 0.1 mg/kg. Blood was sampled for blood glucose concentrations at 0, 1, 2, and 3 hours after injection. Two or

more days later, a higher dose of IGF-1 (0.1 to 0.4 mg/kg twice daily) was initiated and given for periods up to 16 months.

Basal concentrations of total circulating IGF-1 were decreased in 6 subjects but normal in the others. Following the acute injection of IGF-1, the circulating IGF-1 concentration decreased very slowly in most and remained elevated for at least 24 hours. In one patient with leprechaunism, the concentration fell rapidly to baseline within 12 hours. The subjects' plasma glucose levels declined 50% to 87% in the low-dose study and 45% to 87% in the high-dose study. Circulating plasma insulin levels fell in response to IGF-1 in a majority of patients. Two patients experienced side effects, including nausea and pallor in one patient and headache in the other.

IGF-1 levels in the long-term study remained at supraphysiologic levels except in 1 of the patients with leprechaunism. Daily plasma glucose profiles demonstrated reductions in both fasting and postprandial levels. In addition, hemoglobin A_{1c} and fructosamine concentrations decreased

during IGF-1 treatment. Plasma insulin and C-peptide levels decreased in many of the patients, including those with leprechaunism. Surprisingly, acanthosis nigricans improved slightly in some of the patients. In the 1 patient with high concentrations of plasma testosterone, the degree of hirsutism did not change during therapy. No hypoglycemic episodes were recorded in any patients.

Background retinopathy occurred in 1 patient with congenital generalized lipodystrophy 1.5 months after onset of treatment. Weight gain occurred in some, including those with leprechaunism. One patient with leprechaunism had an increase in subcutaneous fat mass, and the other had an improvement in skin elasticity and in linear growth. Signs of acromegaly did not occur during the limited long-term therapy. Low titers of IGF-1 antibodies were detected in 3 of 8 patients.

These results show that IGF-1 can be used clinically as a hypoglycemic agent in some patients with extreme insulin resistance and in whom insulin is not effective. Seven of the 11 patients (5 with type A syndrome and 2 with leprechaunism) had defective insulin receptor functions. The authors postulated

that IGF-1 may have exerted its blood glucose-lowering effects through its own receptors.

Kuzuya H, et al. *Diabetes*. 1993;42:696-705.

Editor's comment: *This limited study presents another potentially important clinical use of recombinant IGF-1. Previous therapy, including massive doses of insulin, proved ineffective in controlling hyperglycemia and/or hyperinsulinemia in these patients. The authors point out that IGF receptors are present in high concentrations in muscle but in minimal concentrations in adipose and hepatic cells. Thus, they hypothesize that the glucose-lowering action of IGF-1 may be primarily by its own receptor, inducing glucose transport and stimulating glycogen synthesis. IGF-1 also has been shown to inhibit pancreatic beta-cell function in isolated perfused pancreatic preparations. More sophisticated acute and long-term studies of IGF-1 in these types of disorders are needed. These initial findings, however, are exciting and encouraging.*

William L. Clarke, MD

Failure to Improve Height Prediction in Short Stature Pubertal Adolescents by Inhibiting Puberty With Luteinizing Hormone-Releasing Hormone Analogue

Treatment with long-acting D-Trp6-luteinizing hormone-releasing hormone at a dose of 3.75 mg IM monthly was given for 24 months to 17 endocrinologically normal adolescents of short stature (9 females ages 11.8 ± 1.5 years; 8 males ages 13.2 ± 1.1 years). The patients were referred at pubertal stages II-III according to Tanner, with a height prediction below -2.5 SD according to Bayley and Pinneau tables.

Pubertal progression was suppressed during the 2 years of analogue therapy, but then resumed shortly after the end of treatment. Annual growth rate remained in the prepubertal range during the treatment period and did not increase with the resumption of sexual development. A reduced rate of bone maturation was observed during the 2 years of analogue treatment, but there was no clear-cut increase of the height to bone age relationship at the end of treatment nor after the post-treatment observation period of 12 to 14 months. Thus, after approximately 3 years of study, no significant improvement of predicted adult stature was obtained. There were no side effects, but psychological problems occurred mainly related to the failure to increase height.

The authors conclude that even if methods for predicting adult height are not accurate, the data suggest that use of LHRHa in endocrinologically normal short subjects entering puberty at normal age with a poor height prognosis does not offer enough possible advantages on growth to offset the psychological drawbacks, and this approach cannot be considered as routine treatment in this situation.

Linder D, et al. *Eur J Pediatr*. 1993;152:393-396.

Editor's comment: *Further studies which are undertaken under rigid protocol are needed to answer the question whether LHRH analogues can be effective in normal short individuals. I suspect that the results of such studies will confirm the data presented here as GH production falls significantly when LHRHa is given. This article is abstracted because it is one of the first to report attempts to increase growth in normal length and intrauterine growth retarded children by using LHRHa. Ten of the subjects had a birth length >-2SDs below the mean height.*

Robert M. Blizzard, MD

Preliminary Study of the Efficacy of Insulin Aerosol Delivered by Oral Inhalation in Diabetic Patients

In this study the efficacy of orally inhaled insulin in normalizing plasma glucose levels during the fasting state in noninsulin-dependent diabetes mellitus (NIDDM) patients was investigated. The subjects were nonobese, nonsmoking NIDDM volunteers aged 35 to 62 years. Body mass index ranged from 19.29 to 27.21, with a mean of 23.94 ± 3.00 . The patients had never received insulin therapy. Five were on oral antidiabetic medication, which was discontinued 2 to 4 days before insulin inhala-

tion. An insulin dose of approximately 1.0 U/kg of body weight was administered as an aerosol by oral inhalation. Aerosol was generated by a raindrop nebulizer from 2 mL of undiluted regular 500 U/mL pork insulin solution. During the aerosolization procedure, the nebulizer was activated 6 times in quick succession. Insulin aerosol generated during these 6 actuations accumulated in the holding chamber. After the sixth firing, aerosol in the holding chamber was inspired as a bolus through

a mouthpiece with a flow rate of 17 L/minute. The small particle size produced by the raindrop nebulizer, the low regulated flow rate, and the holding chamber were employed in combination to minimize impaction and loss of insulin in the mouth. Insulin units available at the mouth were quantified by adding radiolabeled technetium Tc 99m pertechnetate to the insulin solution. Radioactivity deposited on the filter attached to the mouthpiece was counted and found to represent $27\% \pm 4\%$ of the initial dose of radioactivity that was delivered into the holding chamber. Twenty-seven percent of 24 U of insulin in the chamber (6.48 U) was the amount of insulin available for inhalation at the mouth after 6 actuations. Thus, to deliver a total of 1 U/kg, volunteers inhaled 8 to 13 times from the holding chamber, depending on their body weight. The percentage of deposition in the lungs of each subject was also quantified from a gamma camera scan of the anterior chest after inhalation of a radioaerosol. Quantitative analyses of the lung scans showed that the deposited fraction ranged from 50% to 93% of the inhaled dose. Mean deposition below the larynx was $79\% \pm 17\%$. The remainder was deposited in the oropharynx or was exhaled.

After inhalation, plasma glucose and insulin concentrations were monitored while a saline drip was continuously administered intravenously. During a second 12-hour fasting period on another occasion, 3 of the subjects inhaled placebo aerosol generated from 0.9% saline.

The mean plasma insulin level increased from 11.8 $\mu\text{U/mL}$ to 44.8 $\mu\text{U/mL}$, with an average time $\pm\text{SD}$ to peak level of 40 ± 34 minutes. The plasma glucose levels decreased from a mean of 225.5 ± 46.3 mg/dL to normal levels of 70 to 115 mg/dL in 5 of the 6 diabetic subjects. In all subjects, maximum percentage decrease from baseline ranged from 43% to 71%, with a mean $\pm\text{SD}$ of $55\% \pm 10\%$. Average time $\pm\text{SD}$ to the lowest glucose level was 153 ± 27 minutes. In contrast, the mean reduction in glucose levels after placebo inhalation in 3 subjects was $13\% \pm 9\%$.

No pulmonary adverse effects or hypoglycemic symptoms were noted. These preliminary results suggested the feasibility of controlling plasma glucose in diabetic subjects during the fasting state by oral inhalation of insulin aerosol. It was pointed out that the time required for the peak insulin level was variable between subjects ($\text{CV} = 85\%$). Nevertheless, this variability did not appear to significantly affect time to maximum decrease in plasma glucose level between subjects, which was more predictable (ranging from 120 to 200 minutes) with a CV of 18%.

Laube BL, et al. *JAMA*. 1993;269:2106-2109.

Editor's comment: This is an exciting and very well done study that proves the short-term efficacy of inhaled insulin in reducing plasma glucose levels in NIDDM patients. The authors of this study were able to overcome the difficulties of delivering insulin to the lungs that plagued previous researchers in this field (Wigley et al. *Diabetes*. 1971;20:552; Elliot et al. *Aust Paediatr J*. 1987;23:293) as well as the adverse signs or symptoms that occur with nasal inhalation of insulin (Moses et al. *Diabetes*. 1983;32:1040; Salzman et al. *N Engl J Med*. 1985;312:1078).

Inhaled insulin offers a safe and painless mode of insulin delivery that possibly could completely eliminate insulin injections in the treatment of NIDDM. In addition to avoiding shots by using aerosolized insulin, it may be possible to administer more frequent medication to diabetics and, therefore, allow them more flexibility in daily life while attaining better control of the disease. Of course, more studies, including those utilizing insulin-dependent patients, are necessary to derive more information about the kinetics, action, and metabolism of inhaled insulin. Also, more information is needed to ascertain if there is a difference in allergic reactions or other complications between inhalation and injection modes of therapy, as well as long-term safety and efficacy of this new treatment modality.

Clinical proof of practicality of inhaled treatment may open a new horizon in endocrinology. The authors have raised the possibility that other peptide hormones currently administered by injection, such as growth hormone and calcitonin, might also be effectively delivered as an aerosol through the lungs.

Fima Lifshitz, MD

2nd Editor's comment: Laube and colleagues have presented some intriguing new information concerning an alternative method of insulin delivery. Clearly their work is an advance over earlier studies of inhaled insulin, but should be regarded as very preliminary. No information is given regarding insulin kinetics (onset of action, duration, etc.) nor the actual patient time involved in administering the 8 to 13 inhalations necessary to deliver 1 U/kg. Reductions in blood glucose ranged from 43% to 71%, demonstrating tremendous variability in the biologic activity of a dose that is standardized when it leaves the inhaler. Such variability increases the potential for both severe hypoglycemia and recurrent hyperglycemia. In addition, frequent administration of short-acting insulin would not obviate the need for long-acting insulin to prevent fasting ketosis and the hyperglycemia associated with the dawn phenomena. Finally, experience in treating children and adolescents who have asthma with inhaled corticosteroids and beta-adrenergic agents suggests that the accuracy of inhalation treatment relies heavily on technical skills that are at best variable. Thus, it is unlikely that such therapy would be a practical tool for achieving near-normal glucose levels.

The need for more convenient, less painful methods of insulin delivery is clear. The Diabetes Control and Complications Trial (GGH. 1993;9:3) results suggest that intensive therapy can be associated with a reduction in the microvascular complications of diabetes. The majority of subjects receiving intensive therapy in that study injected insulin 3 or more times daily. Any method that might facilitate frequent insulin administration could be of potential benefit to persons attempting to achieve near-normal blood glucose levels. It would appear, however, that inhaled insulin as described in the present paper might lead to such a variable effect as to be of little value at the present time.

William L. Clarke, MD

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Prader-Willi Syndrome: Consensus Diagnostic Criteria

Because of the difficulty in identifying patients with Prader-Willi syndrome (PWS), the authors developed a list of major and minor diagnostic criteria and supportive findings (see Table 1 and Figure 1) and a scoring system designed to aid in this effort. Each major criterion is assigned 1 point and each minor criterion a 1/2 point; supportive criteria are assigned no value. To establish the diagnosis of PWS in a child <3 years of age, a point score of 5 (at least 4 from the major group) is required, and for a subject >3 years of age a point score of 8 (at least 5 from the major group) is required.

Holm VA, et al. *Pediatrics*. 1993;91:398-402.

Editor's comment: This reviewer has found that one of the most difficult aspects of making the diagnosis of PWS has been to consider this disorder in the differential at the very beginning. Recognition of this disorder is particularly hard in early infancy

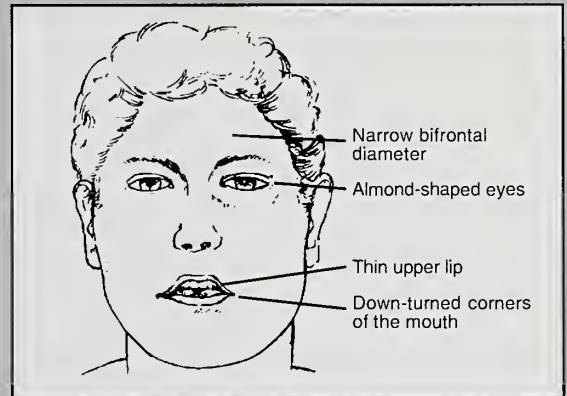
Table 1

Diagnostic Criteria for Prader-Willi Syndrome

- I. Major Criteria (1 point each)
 - A. Hypotonia in the neonatal period
 - B. Failure to thrive in infancy and early childhood
 - C. Rapid weight gain after 1 year of age
 - D. Characteristic facial features (see Figure 1)
 - E. Hypogonadism
 1. Small phallus, cryptorchidism (male)
 2. Delayed gonadarche
 - F. Developmental delay
 - G. Hyperphagia; aggressive food-seeking behavior
 - H. Deletion of 15q11-13 or evidence of maternal disomy
- II. Minor Criteria (1/2 point each)
 - A. Decreased in utero activity
 - B. Behavioral abnormalities: temper tantrums, violent outbursts, obsessive-compulsive, rigid, argumentative, oppositional, stubborn, lying (5 or more required)
 - C. Sleep disturbances/apnea
 - D. Short stature (relative to bone age)
 - E. Hypopigmentation
 - F. Small hands/feet
 - G. Narrow hands with straight ulnar border
 - H. Esotropia, myopia
 - I. Viscous saliva
 - J. Articulation difficulty
 - K. Skin picking
- III. Supportive Findings (no points)
 - A. High pain threshold
 - B. Decreased vomiting
 - C. Temperature instability
 - D. Scoliosis/kyphosis (in the second decade)
 - E. Early adrenarche
 - F. Osteopenia
 - G. Skilled at jigsaw puzzles
 - H. Normal neuromuscular studies

Adapted with permission from Holm VA, et al. *Pediatrics*. 1993;91:398-402.

Figure 1
Facial Features in Prader-Willi Syndrome



Reprinted with permission from Holm VA, et al. *Pediatrics*. 1993;91:398-402.

when a myriad of causes may result in hypotonia and failure to thrive. In the male infant with small phallus or cryptorchidism, this diagnosis comes more readily to mind, but in the hypotonic female infant the diagnosis is more difficult, even with standards for clitoral size, which are primarily useful for identifying the enlarged, rather than the small, clitoris. As the hypotonia improves, hyperphagia develops and the older infant or young child becomes obese. Therefore developmental delay becomes apparent and the diagnosis becomes evident as well.

The diagnostic criteria set forth by Holm et al are indeed useful, particularly for general pediatricians, who initially see these patients. In the experience of this editor, these criteria and scoring system have been employed to rule out PWS in obese, developmentally delayed children who are often referred with possible PWS. However, rigid adherence to these criteria is to be avoided and clinical judgment trusted when a given point score is not realized at any one time during the course of this disorder. Further observation will often clarify the situation.

The criteria also point out some important aspects of PWS, including the increased occurrence of premature adrenarche and osteopenia, which is accompanied by a propensity for fracture with minimal trauma. Mention is made of growth hormone therapy for short stature associated with PWS, but that is currently a subject of investigation and significant controversy.

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MEETINGS CALENDAR

March 13-15, 1994 "Genes in Development and Cancer" March of Dimes Clin Genet Conf, Kissimmee, FL. Info: C Blagowidow. Tel: 914-997-4552; Fax: 914-997-4560.

March 15-17, 1994 Amer Coll of Med Genet, 1st Ann Mtg, Kissimmee, FL. Info: E Strass. Tel: 301-571-1826; Fax: 301-530-7079.*

April 10-14, 1994 Intl Mtg on Sex Hormones & Antihormones in Endo-Dependent Pathol: Basic and Clin Aspects, Milan, Italy. Info: Drs M Motta/M Serio. Tel: 39-2-2940-6576; Fax: 39-2-2940-4927.

April 20-23, 1994 1st Postgrad Clin Endo Course of the Euro Fed of Endo Soc, Gerona, Spain. Info: Prof FF Casanueva. Fax: 34-81-572-121.

April 28 - May 1, 1994 3rd Ann Mtg & Clin Cong of the Amer Assn of Clin Endo, New Orleans, LA. Info: L Kepner/A Jones. Tel: 904-384-9490; Fax: 904-384-8124.*

May 2-5, 1994 APA/APS/SPR Ann Mtg, Seattle, WA. Info: D Anagnostelis. Tel: 708-427-1205; Fax: 708-427-1305.

May 25-27, 1994 4th Intl Mtg of Endo, Rome, Italy. Info: Serono Symposia. Tel: 39-6-442-91-229; Fax: 39-6-442-91-324.*

June 1-4, 1994 1st Intl Mtg of the GH Research Soc, Aarhus, Denmark. Info: Drs J Christiansen/J Jorgensen. Tel: 45-86-1255-55/ext 2084; Fax: 45-86-1378-25.

June 8-14, 1994 54th Ann Mtg of the ADA, New Orleans, LA. Info: ADA. Tel: 703-549-1500/ ext 330; Fax: 703-836-7439.

June 17-24, 1994 3rd Eur Cong of Endo, Amsterdam, Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9474.*

June 23-24, 1994 8th Intl Study Group on Diabetes Treatment with Implantable Insulin Delivery Devices Mtg, Nice, France. Info: Prof J Selam. Tel: 33-1-4234-8376; Fax: 33-1-4354-1564.

June 15-18, 1994 76th Ann Mtg of the Amer Endo Soc, Anaheim, CA. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.*

June 22-25, 1994 33rd Ann Mtg of the ESPE, Maastricht, Netherlands. Info: Prof J Van den Brande. Tel: 31-30-32-0521; Fax: 31-30-33-4825.

June 30-July 3, 1994 2nd Intl Cong on Prader-Willi Syndrome, Cambridge, England. Info: Dr B Laurance.*

July 17-24, 1994 3rd Eur Cong of Endo, Amsterdam, Netherlands. Info: P Wittebol. Tel: 31-20-626-1372; Fax: 31-20-625-9574.

August 20-25, 1994 7th Intl Cong on Obesity, Toronto, Can. Info: Univ of Toronto, CME. Tel: 1-416-978-2719; Fax: 1-416-971-2200.

October 30-November 3, 1994 46th Postgrad Assembly of the Amer Endo Soc, Toronto, Can. Info: W Johnson. Tel: 301-571-1807; Fax: 301-571-1869.

November 2-5, 1994 20th Ann Mtg of the ISGD/IDF Mtg, Tokyo, Japan. Info: Prof T Kitagawa. Tel: 81-3-293-1711-212.*

November 6-11, 1994 15th World Cong of the IDF, Kobe, Japan. Sci Info: Prof S Baba. Tel: 81-78-303-0055; Fax: 81-78-302-7303.

June 14-17, 1995 77th Mtg of the Amer Endo Soc, Washington, DC. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.

June 25-28, 1995 34th Ann Mtg of the ESPE, Edinburgh, Scot. Info: Prof Dr W Sippell. Tel: 49-431-597-1626; Fax: 49-431-597-1675.*

1996 78th Mtg of the Amer Endo Soc, San Francisco, CA. Info: C Huck. Tel: 301-571-1835; Fax: 301-571-1869.

Spring 1996 34th Ann Mtg of the ESPE, Montpellier, France. Info: Prof C Sultan.*

June 5-7 1996 22nd Ann Mtg of the ISGD, Pittsburgh, PA. Info: Prof AL Drash. Tel: 412-692-5851; Fax: 412-692-5960.*

July 20-25, 1997 16th Cong of the IDF, Helsinki, Finland. Info: Prof M-R Taskinen. Fax: 358-0-411-244.

July 1997 23rd Ann Mtg of the ISGD in conjunction with the IDF Mtg, Helsinki, Finland. Info: Prof H Akerblom. Tel: 358-0-471-2701.

June 22-26, 1997 5th Jnt Mtg of the LWPES/ESPE, Stockholm, Sweden. Info: Prof Dr W Sippell. Tel: 310-825-6244; Fax: 310-206-5843.*

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